Histochemical studies of Lipofuscin Pigment (Age Pigment) in gut of Male and Female " CHANNA PUNCTATUS " at different age levels.

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DEDICATED TO MY PARENTS

(Mrs. & Mr. Tajammul Hasan)


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DECLARATION.

I hereby declare that the Exceptions of the guidance and Suggestions received from my Supervisor, Dr. R.C. Gupta , Reader, Department of Zoology, Bipin Bihari P.G. college, Jhansi; this thesis is my own Unaided work carried out in the post graduate Department of Zoology, Bipin Bihari, P.G. College, Jhansi, U.P.

I further declare that this is an original piece of my research work and has not been Submitted any where else.

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CERTIFICATE.

This is to certify that the thesis entitled " Histochemical Studies of Lipofuscin Pigment (age Pigment) in gut of male and female " CHANNA PUNCTATUS " at different age levels ; " is an Original piece of research work, carried out by Mrs: Aliya Aijaz, in the Deptt. of Zollogy ; Bipin Bihari, P.G. College ; Jhansi. She has worked under my guidance and supervision for the period required as under para 8 of the Ph.D. Ordinanace. In my Opinion, this thesis fulfils the requirements of the Ordinance, relating to the Ph.D. Degree of Bundel khand University Jhansi.

Date, November, 1999.

(R.C. Gupta.)

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CHAPTER - 1

INTRODUCTION

INTRODUCTION.

Aging is a definite time course and direction for the individual changes in various parts of an orgainsm. It an universal phenomenon in all the Organism, the total of which results in the failure of individual to with stand the stress of his environment. A consistantly noted change in cells composition during aging is the increase of substance variously known as aging pigment, age pigment, lipofuscin, ceroid or wear and tear pigment. Obvious menifestation of aging includes wrinkling of skin, slowness of movement & inability of the eyes to accommodate for near vision. Another menifestation of aging is the wide spread accumulation of pigment granules within the cytoplasm of cells of many orangs particularly in neurones, skeletal myocardial, carpus luteum, spleen, liver and nerve cells. The Structure and composition of pigment may vary form species to species and tissues to tissues.

The progressive accumulation of lipofuscin is considered to be the most indicative. Fluorescent pigment granules were found in the electric lobes of Torpedo, (Lerma & Ventra, 1956), and these were later associated to lipofuscin pigments; (Totaro, 1977). Koneff (1886) was the first to associate the presence of pigment granules with cellcular aging. This hypothesis is now universally accepted so much, so that the cytological modifications corelated to neuronal aging.

Occurrance of lipofuscin pigment was known as early as the end of the last century. Since then, the origin and possible function of Lipofuscin granules have been the subject of much intrest. Histochemical studies in Situ, fluorescent microscopy and recently electron microscopy have yielded no definite conclusin regarding its origin. However, the Origin of lipofuscin has been

attributed to many of the cytoplasmic organelles including mitochondria, golgi apparatus, endoplasmic reticulum and lysosomes, (Toth, 1968).

The most regular cytological change corelated with aging is the accumulation of a type of pigment of lipid origin commonly known as lipofuscin (Bensley, 1947) Numerous investigators have observed accumulation of yellow brown pigment in certain body cells of various aging animals.

The most obvious and consistent evidence of the aging process at the cellular level is the accumulation of lipofuscin pigments. Although there have many studies on the composition and origin or these aging or wear and tear pigments much is yet unknown of lipofuscin real link to the process of cellular aging.

Hueck (1912) reported the occurrence of fluorscent inclusion in cells which generally reforred as lipofuscin or age pigment. Pinkerton (1928) suggested the importance of unsaturated lipids in the biogenesis of lipofuscin and Muhlmann (1910) Stress the significance of pigmentation in nerve cells with age. The involvment of this pigment in aging process has been the subject of much study and controversy. The pigment has been recognised as a distinct intracellular structure for over a century, (Hannover 1842, Koneff 1886). Hannover (1842) observed to these pigments in dissected nerve cells whereas Koneff (1886) reported that the amount of these pigment granules in nerve cells are related to the age of individual. Hodge (1894) reported pigment accumulation in the cytoplasim of neurones of senile individual as well as in honeybee and human nerve cells. Stubel (1911) observed these pigments particularly in brain.

Another possibility may be the involvement of endoplasmic reticulum in formation of lipofuscin. (Chance & Williams, 1954, Kumamoto and

Bourn, 1963) and the foremost Mitochondrial Origin of the pigment was strongly disputed by bondareff (1957, 1959), who suggested a possible releationship of pigment to the golgi Apparatus. Gatenby & Mausa (1950) and Getenby (1953) established a morphological relationship between the golgi apparatus and lipofuscin.

It is known for over a century, that the cytoplasm of aging nerve cells of human and other animals species contain golden brown pigment inclusions called lipofuscin (Whiteford & Getty, 1966).

Mitochondria generally found in association with the pigment and electron micropraphs of dorsal root ganlia from aged mice have been indicated the possibility of pigment formation from them (Duncan et al ; 1960), In a study of neurosecretion in birds (Ghosh et al ; 1962) have obtained some interesting electron micrographs of mitochondria and lipofuscin which further support mitochondrial origin of age pigment. Examinations by light and electron microscopy of human myocardium have revealed that mitochondria can be transformed into granules of lipofuscin (Kooba et al; 1978). Certain other workers have reported that lipofuscin is derived from degenerating mitochondria (Hess ; 1955, Glees and gopinath; 1973, Spoerri and Glees; 1973, Gopinath and Gless; 1974).

Lipofuscin is general term assigned to fluorescent material that accumulates in cells as they aged, while reports on the fluorescent properties vary; this material typically emits light between 450 nm and 600nm. Fluorescence discribed to lipofuscin has been observed in post mitotic cells from a variety of organisms within intracellular granules composed, in part, of protein and lipid. (Totaro, Gless & Pisanti. 1985; Zs Nagy, 1988; Tsuchida , Miura & Aibara, 1987; Harman, 1991, Porat, 1991 L. Because of its age related increase and

Seemingly universal occurrence lipofuscin is considered a hall mark of aging.

Support for a mitochondrial source of age pigment or lipofuscin, however clashes to some extent and several groups of workers have suggested an identity of lipofuscin with lusosome. De Duve et al (1955) provided concerning evidence for Structural and cytochemical identification of lipofuscin with lysosome in nerve cells. According to Samorajski et al (1964) lipofuscin represents the remains of lysosmes. Finding of Gedigk and Bontke (1956), Essener and Novikoff (1960 Hassan and Glees (1972) and Brunk and Ericsson (1972) are particularly relevant to this hypothesis.

The complexity of the pigment is evidenced by voluminous litrature which has accumulated during this period. According to Conner (1928), two groups of pigments accumulate in the body with age namely lipochrome and haemochrome. The former is exogeneous in origin and is found normally in such organs as the adrenal cortex, carpus leteum, liver , spleen and skin, while later is the wear and tear pigment and has been found in many organs including heart.

Some of the pigment granules found in the various tissues have been thought to owe their origin to the process of aging. This assumption seems to be justified since pigments are not normally found in young animals but are seen in the tissues of old animals. Publications by Bjorkerud (1964), Strehler (1964) and Goldfisher, et al (1966) reported the informations available concerning the biochemical Composition, histochemical properties and the Ultra structural morphology of these pigments.

Lipofuscin has been located in cytoplasm in the groups of various sizes and also collected from the perikaryon or at the poles of cells. Histochemical studies (Bourne, 1973) have clarified, but not defined the

composition of lipofuscin while the genesis and functional significance of these masses of pigments are still a matter of discussion. It is widely held that these granules are either of a mitochondrial (Miquel, 1971; Gopinath & Gless, 1974; Hasan & Spoerri, 1974) or a lysosomal (Samorajski & Ordy, 1972 ; Hasan & Glees, 1972, 1973) derivatives. Regarding the functional significance it is suggested that lipofuscin is cellular debris waiting to be eleminated. (Gless and Hasan, 1976).

The term lipofuscin was proposed by Borst (1922), however other terms that have been assigned to the insoluble yellow intracellular material are, lipochrome (Findley; 1920, Bourne, 1934, Fekete, 1946, wear and tear pigment (Hemperi, 1934, luleolipin (Rossman, 1942); caroid (Reagan, 1950), lemofuscin (Jayne; 1950), yellow pigment (Hyden & Lindstrom; 1950), lipogene pigment (Gomori, 1952,) lipopigment (Gedigk & Bontke, 1956), age pigment (Strachler et al, 1959, Brody; 1960) and lipid pigment (Samorajski & Ordy; 1967), but the term lipofuscin has been accepted by several investigators (Bensley; 1947, Strechler et al ; 1959, Pearse; 1960, Frank & christensen; 1968).

Lipofuscin is mainly lipoproteic in nature it increases with age so much so that it is known as " SENESCENCE PIGMENT". (Bourne, 1973; Gless & Hasan, 1976). Lipofuscin is widely believed to be a high molecular weight material generated upon Oxidative damage of cellular components. (Tsuchida, Miura & Aibara, 1987; Kikugawa & Beppu, 1987; Harman, 1990; Porta, 1991; Ivy et al , 1991). Bensley (1947) Demonstrated the pigments in the mitochondria of guinea pigs liver cells and compared these with those produced by the autooxidation of phospholipid and unsaturated fats.

Lipofuscin pigments accumulation has been observed to

increase with age in human being (Brody, 1960; Samorjaski et al, 1964), dogs (Whiteford & Getty, 1966, Few & Getty, 1969;), Pigs (Whiteford * Getty, 1966; Few & Getty, 1967; Nanda & Getty, 1971), mice (Samorajski et al , 1968), rats (Reichel et al , 1968, Brizzee & Johnson, 1970 & monkeys (Brizzee et al , 1974). Mostly these studies has revealed the semiquantitative evidences for lipofuscin, increase with age, whereas Strehler et al, (1959) & Goyal (1981) reported quantitatively that lipofuscin pigment granules accumulate linearly with time in human myocardium.

Lipofuscin has been proposed as a basic " LAW OF SENESCENCE" for cellular aging (Strehler & Barrow, 1970). However the early appearance and experimental modifications of lipofuscin by druge, hormones, antioxidants and immunoregulators have suggested to others that the pigments may represents a harmless or innocuous by product of cellular metabolism. (Nandy, 1968; Kormenday & B ender, 1971).

This material has been noted in a variety of cells over a period of a century or more on research (Strehler et al , 1959; Samorajski et al, 1964; Bourne, 1973. One of the most consistent morphological observation made on tissue from aging animals is the accumulation on the intra cellular aging pigment (Timiras, 1972. Schofield and Davis 1978;).

The most typical lipofuscin pigment is desoribed by (Pearse, 1960) as a brown deposite which is strongly basophilic and contains reducing moieties. By histochemical staining techniques and, light and electron microscopy an increase in pigment content with age has also been noted in the nervous system of mice (Samorajski, Keefe & Ordy, 1964; Samorajski, Ordy & Keefe, 1965) dogs (Sulkin, 1955; white ford & Getty, 1966), pigs (Whiteford & Getty

, 1966) and human (Hampert, 1934 ; Humphery, 1944; Sulkin, 1953; Issdorides & Shanklin, 1961; Samorajski, et al. 1964); rats (Monji et al 1993). Although there have been only a few studies on the effect of dietary Vitamine E on lipofuscin accumulation with age in the rats brain, the results have been inconsistent. (Katz, et al 1984; Sarter, 1987; Sato, 1988). Studies indicated that the rate of formation of lipofuscin pigment is increased in mice and rats by a Vitamine E deficient diet. (Sulkin & Samoraski, 1960; Reichel et al , 1968). Whereas Supplementation of diet Vitamine E decresed lipofuscin content in mice brain. (Freund, 1979). Vitamine seems to play an important role in the central nervous system with regard to metabolic stability and physiological functions (Nelson et al 1981).

By using fluorescent light microscope (Munnell & Getty, 1968) determined strong corelation between age and the amount of lipofuscin in myocardium of human and dogs. The granules accumulated faster in dogs than human, whereas the life Span of later is more than that of the former. Munnell & Getty (1968) suggested that this point has a meaningful connection between lipofuscin and life span. The linear realtionship of accumulation of lipofuscin is supported by (Strechler et al , (1959) in man but children below 12 years of age did not possess the pigment. Quantitative observations made by (Goyal, 1981) also showed linear relationship between fluorescent accumulation in myocardium with age in man.

Although various investigations have attempted to elicit the origin and function of lipofuscin. Results have implicated almost every cell organelle, that the accumulation of lipofuscin in non replaceable, fixed post mitotic cells is n age core lated process, is well established. (Jaune, 1950; Strehler et al, 1959; Brody, 1960). However it is indeed questionable, if lipofuscin is ab age pigment

resulting from a genetic program or the result of enviromental influences i. e. bilogical noise. Research studies of lipofuscin have emphasized phyletic distribution, occurrence in human tissues, biophysical and biochemical properties, histochemical affinities and ultra structure.

Lipofuscin is known to accumulate in many tissues with increasing age of an orgaism as specially shown by histo chemical satinig, under light and electron microscope, (Samorajski & Ordy, 1967; Brody & Vijai Shanker, 1977;) An extensive review by Szabo *1935) gives descriptions of age related morphological changes in many invertabrates from protozoans to insects, including the accumulation of age pigment as one indication of aging in these lower forms. Szabo (1935) also describe the accumulation of age pigment in ganglia and nerve cells of many differenrt speices of molluscs. Rudziansk (1961) reports this age pigment in protozoans cytoplasm, Epste in et al (1972) in namatodes, totaro (1981) in Apysia, limacina, Sheehy et al (1991) in insects, crustacenas and other aquatic species and Fleming et al (1992) in Drosophilla.

Gupta (1985) has studied the lipofuscin pigment accumulation in heart, midgut and malpighian tubule of male insect. Agarwal (1995) has also studied the lipofuscin pigment accumulation in brain and heart of Chana punctatus.

Lipofuscin pigment accumulation in the human heart has been observed to increase with age (Strehler et al, 1959); Mc Millan & Lev, 1962; Andrelej & Buozynski, 1972) reported an absence of pigment in the myocardium of person below years of age. with the advancement of age lipofuscin pigments has been observed to increase Significantly in the ventricle of rat, (Reichel, 1968) and dog (Munnell and Getty 1968). There is no significant report available on the

rate of Lipofusscin pigment accumulation in human myocardium except that of Strejler et al, (1959).

Tissues of aged animals, particularly nervous tissues, adrenal gland, cardiac muscles and Skeletal muscles are known to contain substantial amount of Lipofuscin or age pigment. (Jayne, 1950, 1959, 1963; Planel & Guilhem, 1955; Mayers & Chariaper, 1956; Strehler et al , 1959, Wilcox, 1959; Mac Kinnon & Mac Kinnon 1960, Tcheng et al, 1961 et al 1961; Whiteford & Getty, 1966).

The mejority of investiagtors studying caridac lipofuscin have been reported on its increasing incidence with age (Bohmig, 1935, Jayne, 1950; Strehelr et al, 1959 measured the amount of age pigment in microscopic section from hearts of humans, the study showed that the accmulation of the pigments increased lineraly with age. Studies from Laboratory have been directed towrard the relationship of lipofuscin to aging (Whiteford & Getty, 1966; Few & Getty 1967).

Gardner (1940) Considered the presence of pigment in spinal ganglion cells to be related to age. Sulkin and Kuntz (1952) found the sympathetic ganglion cells to contain pigment granules in childrens and adults upto go years of age, but they are unable to demonstrate a progressive increase in pigment consentration in human sympathetic ganglion cells with age. However, the presence of this material was frequently observed in dogs after 12 years of age, although pigments were not present in a Series of dogs aged 30 dasy to 9 years. On this basis these authors considred pigmentation to be definitely associated with aging is these animals.

From the electron micrographs it has been determined that

lipofuscin appears as clusters of high density and complex ultrastructure consisting of myelin like figures arrange in several configurations within a single body. (Samorajski, et al, 1964; Samorajski, Ory & Keefe, 1965). The pigment bodies were reported to range in size from 2-3 microns were vocoulated, and were surrounded by a single limitig membrane (Samorajski et al, 1964; 1965). Observations with light microscope on the morphological featrues have shown the pigments as round or oblong granules increasing in size with age. (Brizzee et al, 1969.

Occurrence of interneuronal lipofuscin is established to be ange associated chage in fishes, amphibians, birds, rats pigs, dogs and man (Wilcox, 1959; Sulkin 1961; Whiteford & Getty . 1967; singh & Mukerjee 1972, Lopez et al 1993, Girven et al; 1993, Kara 1994). Whitefrod & Getty (1966) and Nanda & Getty (1971, 1973 (reported the occurrence of lipofuscin and its increase amount with age the nculeus, Occulomotorii of pigs and dog. Hopker(1951) reported the presence of this pigment in the denate nucleus of the man. Large quantities of lipofuscin have been found in the central nervous system of Torpedo particularly in the electric lobes which are ganglia that regulate the function of electric organs, (Totaro & Pisanti, 1981).

Studies on the intra-cellular distribution of the pigment showed different pattern during aging. Hopker (1951) noted a variable distribution pattern relative to certain cell types and aging, whiteford and Getty (1966) grouped the intracellular distribution of the pigment in various areas of the brain of dogs and pigs in to four categories :- (a) Diffuse type granules, Small and evenly scattered throughout the Cytoplasm; (b) perinulcear pigment clusters, usually concentrated

in a crescent shaped at or near the axon hillock;

(d) Bipolar aggregation

Cellular and regional differences have been reported in the accumulation of lipofuscin in the brain of man (Brody, 1960; Braak; 1971; Dayan, 1971). These findings have suggested that the deterioration of some sensory associative and motor function in sonescence may be a consequence of selective alterations in discrete regions of the brain rather than uniform cellular aging throughout the nervous system.

The accumulation and changing pattern of distribution of lipoid pigment Bodies in neurones of the mammalion nervous system has been described by many investigators, (Dolley, 1911; Harm, 1924; kuntz, 1938; Nandy & Bourne, 1966). Over a period of more than 80 yeras; among recent investigations Brody (1960) published data on the relative amount and pattern of distribution of lipofuscin in neuroes. Neurones exhibits lipofuscin pigment in both scatteredand congregated distribution. Lipofuscin pigment diffused throughout the cytoplasm in early stages byt may later be localized in perinuclear clusters or in polar aggaregate of cells. (Brizzee, el at ; 1969).

A number of studies on the aging pigment in nervous tissues with regard to its occurrence , accumulation with age, its origin and significance, have been published and these have been review in detail by (Lansing, 1952; Birren, 1959; Bourne, 1961; Barka and Anderson, 1963; Whiteford, 1964; Few, 1966 and Nanda, 1970. The available records show that the occurrence of aging pigment in nerveous tissue has been studied exttensively in rat, mouse and guinea pig (Wilcox, 1959). Several studies have been reported on human, (Andrew. 1956); bondareff, 1959; Bourne, 1961) dog, (Dolley, 1911; God Pasture, 1918; Harms

1924; Sulkin & Kuntz, 1952; Sulkin, 1953, 1955a, 1955b), where as only few studies available on the aging of nervous tissues of the pig, (Whiteford, 1964; Few, 1966).

Lipofuscin accumulaltion with aging malnutrition and under Various experimental Conditions in the post mitotic cells of experimental animals and has been extensively studied and reviewed, (Glees & Hasan, 1976; Brizzee & Only, 1981; Patro, Sharma & Patro, 1988).

Whether Lipofuscin is a harmful agent or an inert or harmless product is a debatable question.

Keeping in view the relationshiop of age pigment accumulation the present study was planned to examine the earliest and gradual age dependent accumulation of lipofuscin pigment in different tissue of prematuare, mature & post mature, Channa punctatus :-

The specifice aims were as follows :-

1. To establish the age at which lipofuscin pigment first appears in different tissues.
2. To examine possible differences in different organs and cytological distribution of lipofuscin pigment in different tissues in regard to the Gut.
3. To establish the progressive increase in the intra cellular pigment accumula tion in various tissues.
4. To determine the morphology of pigment granules.
5. To evaluate the percentage of pigmented cells in Gut of Male and Female at different age levels.

CHAPTER - 2

MATERIALS & METHODS.

Channa punctatus, a live fish is best suited for present investigation as it may be easily maintained under laboratory conditions for longer duration. The availability of channa puncatus is round the year and can be procured on experimantal requirements fro natural habitate.

The fish and reaed fishes were dissected repeatedly in breeding season (July to Sept) to boserve their sexual maturity. The gonads, were taken out and actual state of maturity has been histologically studied in each of fish taken in the Consideration for the present investigation.

Regarding the sexual maturity it is observed that channa puncatus under primitive stage exhibits thin gonads, translucent pale in colour with in conspicuous vascularization. Histologically , the ovary shows immature oocytes, while tests consists of small seminiferous tubules full with speramtogonia in formative phase.

In constinuation when advanced (adult) fishes were examined, the glands were found turgid, thicker, opaque, deep yellowish in colour and a large number of ova and sparms were visible, thorugh ovarian and testicular cortex respectively. The gonads attain their maximum weight and volume, the fishes becomes gravid due to ripe gamets and, abodmen rounded and bulged.

Further when larger and older fishes were dissected, the gonads were found to flacid due to excessive discharge of ova sperms. The weight and volume was considerably reduced. Histologically, the voaries show atretc discharged follicles and tests show empty and collapsing seminiferous tubules.

On the basis of observations the experimantal fishes were

classified into three progressive age groups, i.e. premature ; Mature; and post mature, respectively.

Under the matured Condition fish exhibits morphological distinctive characterstics which were mainly noted during acute maturation of fish, are given as such for acrual determination of fish channa punctatus.

(1) Soft bulging and rounded abdomen.

(2) Bright colouration of the body.

(3) Fishes with prolonged fins & fin rays.

(4) Milt easily cozes out on pressing the abdomen.

FIXATION OF TISSUES.

Studies performed in considering the gut of channa punctatus The fishes from all the three age groups were dissected, and Gut were fixed in 10% neutral formaline (90 :10 water and formaline by Volume). For histological and histochemical observations, representative tissues from different fishes of progressive age groups were fixed in small tubes for 4-6 days. (Brody, 1960; David et al, 1960 ; Nandy, 1968 ; Samorajski et al, 1968).

PREPARTION OF BOLCKS AND MICROTOMY.

After fixing for 4 - 6 days all the tissues were dehydrated in graded alcoholic series in small pieces. After completion of 5 days the material was taken out from the mixture of 10% neutral formlalin and tissues were washed twice or thrice with running water tap and were then transformed in the alcoholic

series. Materials were kept seperately at an interval of 10 minuet in 30, 50, 70, 90% and absolute alcohols, and thereafter they were passed through methyl-Benzoate, 3 changes of 10 minutes each and cleared in Benzene by 2 changes of 5 minutes. Finally, materials were kept in molten-paraffin for 3 changes of 30 minutes at the 60 C and blocks were prepared. Series of Sections were cut at 6 thickness and mounted on glass slides.

For qualitative studies, 25 slides of different tissues were prepared for each group. To determine the percentage of pigmented cells in the mid gut and brain approximately equal size were selected randomly from histologic sections and were examined under light imcroscope. The mena of pigmented cells were calculated after examination of cells from the selected stained slides. For quantitative estimation 20 cells per volume were examined from the Gut.

HISTOCHEMICAL METHODS.

Slides with mounted paraffin sections were deparaffinized in xylene adn passed through the alcoholic series.

Histochemical studies were made in sections sustined with following stains :-

1. Nile Blue A (Lillie, 1956)
2. Schmorls ferric ferricyanide method (Pearse, 1960)
3. Carbol fuchsin, Longs- Ziehl - Neelsen method (Pearse, 1960)

With all the above stains, granules were cahracterized by their affinities where as non granular deposition of cytoplasm remain

unstained. According to Sharma and Manocha (1977), the pigment showed a characteristically differential staining behaviour . If, for instance one granule is moderately or intensively positive to a particular stain, other lying in its vicinity may be totally negative or partially negative or partially positive . The Varieations may be due to differences in the composition of the lipofuscin.

Slides of tissues for different age groups were made by each Stain.

1. NILE BLUE A (LILLIES ALTERNATIVE METHOD).

The Stain was prepared from .05% Nile Blue A, in Solution fo 1% Sulphuric acid. The parrafin sections were deparaffinized and hydrated as usual, then stained fro 20 minutes in .05% Nile Blue A Stain. Stained slides were washed for 15 minutes in running water, mounted in glycerine jelly and examined under a light microscope, Lipofuscin pigment granules appeared as green-blue by this stain. Nuclei stained poorly or not at all.

Due to temporary mount, the lipofuscin granules were photo-graphed immediately, after preparation of slides.

2. FERIC FERRICYANIDE ; (SCHMORL,S) METHOD.

This method was used successfully by sulkin (1955) Samorajski, (1964) and Nandy (1968 , 1971). Ferric ferricyanide solution was prepared by 3 parts of 1% ferric Chloride and 1 part of freshly prepared 1% postassium ferrocyanide. Stain was alway used within 30 minutes after preparation.

The paraffin sections of the mid gut were deparaffinized, passed through alcoholic series and washed in water. Then the slides were immeresed in ferric ferrcyanide solution for 5 minutes, dipped in water 4 or 5 times and counter stained in 1% neutral red for 3 minutes. Stained slides were dehydrated rapidly in alcohol, cleared in xylene and mounted in DPX. Prepared slides of each tissue were examined under a light microscope. Lipofuscin pigment granules appeared dark blue whereas nucleus appeared Red.

3. CARBOL FUCHSIN (LONG- NEELESENS) METHOD.

This method has been used several workers for acid fast Lipofuscin (Sulkin 1955; Few & Gett y1967; Manocha & Sharma 1978 ; Nandy 1971). Carbofuchsin solution was prepared as under :-

Basic fuchsin	10 gm.
Phenol	50 gm.
Alcohol	100 ml.
Distilled water	1000ml.

Paraffin sections of the Gut were deparaffinized and hydrated as usual washed in water and stained in carbol fuchsin solution for 3 Hrs. at 60 C. Sections were washed in running and differetiated in 1% acid alcohol untill the red cells became just faint pink. Then sections were counter stained with 5% toludine blue for 50 minutes, again washed in running water dehydrated in alcohol; cleared in xylene and mounted in DPX.

Lipofuscin pigment granules appeared bright red while nucleus was blue in colour.

Statistical analysis was followed after Snedecor (1957).

CHAPTER - 3

OBSERVATIONS AND RESULTS.

In the present Stydy, the intracellular distribution , morphology and Accumulation of lipofuscin pigment in the Gut of channa Punctatus were investigated in three age group. i.e. premature, mature and postmature respectively.

The pigmented cells were observed in all the tissues (GUT) for quantitative and qualitative studies, Results obtained from the observations revealed the following four categories of cells in relation to the amount and distribution pattern of lipofuscin pigment granules.

CATEGORY - 1	Cells with no pigments.
CATEGORY - 2	In very few cells appearance of pigments in first stages.
CATEGORY - 3	Homogeneous pigment granules Scattered through out cytoplasm of cells.
CATEGORY - 4	Cells with heterogeneous and clusters of pigment granules distributed throughout in the cyto plasm or at periphery of the cells or perinuclear in positions.

Studies in relation to number of pigmented cells, structural morphology and distribution of lipofuscin, showed that intracytoplasmic location of pigment granules varies with age in different tissues and pigmented cells increased with age.

AGE GROUP - 1.

The first age group includes premature Channa puntatus, (in male & female). Several paraffin sections of mid gut were stained with Nile blue A, carbol fuchsin and ferric ferricyanide mathods and were examined for eraly appearance of lipofuscin pigment granules.

Observations of stained sections of Gut of premature Fishes have revealed that the lipofuscin granules did not apprear in cells, (Fig No.1 & 2) Nuclei were rounded and centrally located within the Cells Fig. No.3), and non pigment cytoplasmic substance had no affinities with any stain.

Lipofuscin pigments were not observed in mid gut cells, in premature Male & Female fishes. Similarly the results of Mid gut cells have revealed that the Cytoplasmic substance stains lightly but Lipofuscin granules did not appear in cytoplasm (Fig No. 4,5,6).

Results revealed that the cells of mid gut of premature Male & Female Fishes show no pigment granules. While nuclei were found rounded and centrally placed within the cells.

AGE GROUP - 2.

Male & Female mature channa punctatus were included in the second age group. A comparative histological examination of mid gut cells obtained from the (cultured and natural habitates) fishes of second age group at different age levels, revealed various age associated differences in the accumulation, morphology distribution of lipofuscin pigment in the cytoplasm.

MID GUT .

Several paraffin sections of mid gut were stained and Examined for Lipofuscin of the mid gut at different age levels from mature male fish age group were stained and examined for accumulation, morphology and distribution of lipofuscin granules. The observation revealed that the Lipofuscin pigments were first appeared in the cells of Gut of early mature Male and Female Fishes. (Fig No. 7, 8,).

Lipofuscin pigment stained by the Nile blue A, method appeard as dark blue green grenules (Fig No. 9,10) within the cytoplasm of cells. The pigment contents of the cells was judge by the intensity of stain and the distribution of the pigment granuless within the cells.

Observation of stained sections has revealed a moderate increase in the number of pigmented cells and pigment granules per cells, (Fig No. 11, 12)

Results revealed that some of the cells were found to contain lipofuscin pigment granules, where as other cells had no pigment in the same sections, and pigment accumulation within the cells and number of pigmented cells increased along with dark and blue green prominent pigment granules. (Fig No. 12).

In very many observations it is revealed that as the animal proceeds through the adult age, there is a sharp decrease in the number of cells free, from pigment granules and an increase in the number of cells containig pigment granules. That is why the number of cells containing pigment increases with increasing age, counting of pigmented cells showed that approximately 40-60 % of cells were pigmented in this group of age.

TABLE . 1

Average mean percentage of pigmented cells in mid gut of male & female fishes in different age groups.

Age Group	Tissues	Pimented Cells/Volume	Cells Pimented
		Mean + S.E.	%
Pre mature	mid gut	-	-
Mature	mid gut	8+ 0.28	40%
Post mature	midgut	14+0.35	75%

On observation it is noticed that the number of pigmented cells are more in natural habitate of fishes, than that of cultured (reared) habitate in labotatory. That is due to the environmental factors of Lab: The results revealed that the accumulation of lipofuscin is also effected by the natural conditions of the diet of the organism,, light, humudity, water, air and Lab; environment respectively.

Morpholigical characteristics & distribution pattern of lipofuscin pigments were also examined in mid gut and observed that pigments appeared as rounded and homogeneous (Fig No. 13 &14) in structure & increasing in size with age (Fig No. 9,10,11,12) and lipofuscin granules were irregularly scattered through out the cytoplasm of cells. At this stage two types two types of cells were noted (i.e.) cells containing homogeneous pigments and cells without pigments.

TABLE . 2

Morphological characteristics and distribution of Lipofuscin pigment granules in mid gut in the second age group.

Age Group	Tissues	Morphological Characteristios of Lipofuscin Pigment.	Distribution of Lipofuscin Pigments.
2.	Mid gut	Homogeneous and finely Granular.	Irregularly scattered thorugh out the Cytoplasm.

The Studies of the scond are group showed that the accumulation of lipofuscin pigments vary in different tissues at different age levels. The comparative studies of pigmented cells in the Mid gut revealed that the highest percentage of the pigmented cells was found in the mid gut.

Table no. 2 shows that percentage of the pigmented cells increases with the age of Fishes.

HISTOCHEMICAL OBSERVATIONS :-

Histochemical observations were made of all the age groups. Stained slides of the Mid Gut , at differne age levles were also examined to shoe the chemical nature of Lipofuscin pigment. It was clearly observed that one stain had strong affinities with the pigment, on the other hand same stain had

mild or moderate affinities with pigment of the same tissues at different age levels.

In the second age group lipofuscin pigment granules of Mid Gut had strong affinities with Nile blue A, Stain whereas affinities were moderate with ferric ferricyanide and carbol fuchsin.

TABLE 3.

Histochemical nature of lipofuscin pigment in second age group.

Age Group	Tissues	STAINS :----------		
		Nile Blue A	Ferric Ferricyandie.	Carbol Fuchsin.
2.	Mig dut	+++	++	++

+++ STRONGLY POSITIVE.

++ MODERATELY POSITIVE.

\+ MILDLY POSITIVE.

AGE GRUOP. 3

The third age group includes the post includes the post mature channa punctatus for a detailed study of lipofuscin pigments. Though the previous age groups showed that lipofuscin pigment granules increases gradually and lie scattered in the cytoplasm by the observation of the third age group revealed that the pigment granules have tendancy to aggregate in groups. More over several aggregated groups of pigments granules were observed particularly in senescent cells of mid gut. Neverthless the number of pigmented cells also steadily

increased with age. Different type of accumulation and distribution of pigment granules were also noted in the present investigation.

The microscopic examinations of the mid gut cells revealed various age associated differences in number and distribution of dense pigment granules in the cytoplasm. Variable size and amount of lipofuscin pigments were also observed in the gut cells at different age levles, and Gut cells showed a clear agegragation of pigment granules. Some of the pigment granules which were observed in aggregated forms appeared as complete masses in older age. Such chages show an interesting characterstics of fusion among the pigment granules, however these aggreaged pigment granules were more heterogeneous and more irregular (Fig No. 15,16,17,18,19,20,21,22). Obeservations revealed that as animals proceed gradusIly through old age there is an increase in the number of cells containing lipofuscin pigments and a decrease in the number of cells containing lipofuscin pigments and a dcrease in the number of cells free from pigments. Counting of the pigmented cells showed that approximately 50-85% cells were pigmented in this age group.

TABEL - 4

Average mean percentage of pigmented cells in mid gut in different age groups.

Age group.	Tissues	PIGMENTED CELLS/VOLUME,	CELLS PIGMENTED
		Mean ± S.E.	%
Premature.	Mid gut	----------	----------
Mature	Mid gut	10± 0.63	50%
Post Mature	Mid gut	17 ± 0.26	85%

Morphlogical characterstics and distribution pattern of lipofuscin pigments were also examined . Observations revealed that granules in the younger animals were just visible even by means of the light microscope. As the animals inreased in size the granules enlarged and tended to form clusters and appeared larger in size.

Results obtained from the observation revealed that a greater number of pigmented cells contained hetrogeneous and clumped or clustered pigments instead of dispersed granules in the cytoplasm and the clumped granules generaly scattered inthe cytoplasm of cells. Lipofuscin pigment granules were so abundent that they generally occupied the entire cytoplasmic area of cells, however few pigments were deposited at the periphery of the cells (Fig No. 23,24,25,26,27,28,29,30).

Heterogeneous pigment granules within the cells observed only in this third group. It was also noted that the size, complexity adn intra cellular distributation of the pigmented granules were variable within the cells at different age levels. All the four categories of cells were found in the Mid Gut, during the investigation of third age group.

TABLE - 5

Morphological characterstics and distribution of lipofuscin pigmentes in Mid Gut in different age group.

Age group.	Tissues	Morpholigcal Characterstics of Lipofuscin Pigments.	Distributin of Lipofuscin Pigments.
Premature. Nature	Midgut. Midgut.	No pigments. Homogeneous, appeared tiny to larger in size.	No. pigments Irregularly Scattered Throughout the Cytoplasm.
Post Mature.	Midgut.	Heterogeneous, Majority of cells contain cluped pigment.	Pigments generally Scattered throughout the Cytoplasm, few located at the periphery.

Results obtained from the observation revelaed that lipofuscin pigments were variable in different tissues in different age groups. It was noted that the accumulation and complexity of lipofuscin granules increases with age.

Examinations of histological pigmented cells in the Mid Gut in the Male & Female fishes at the different age levels in (Graph 1.) which shows that increases of pigmented cells with age in all the Tissues.

HISTOCHEMICAL OBSERVATIONS.

In thrid age group, maximum accumulation and similar morphological characterstics of Lipofuscin pigment were observed in Mid Gut. The Lipofuscin granules of Mid of Male & Female fishes had strong affinities with Nile Blue A and ferric ferricy anide while moderately stains with carbol fuchin. Table No. 5 Shows that the pigment granules in Mid Gut re acted strongly and moderately with different stains (i.e.) Nile Blue, A, ferric ferricyanide and carbol fuchin.

TABLE - 6

Showing staining behaviour of lipofuscin pigment granules in the different tissues in third age group.

Age group.	Tissues	STAINS :-		
		NILE BLUE a.	FERRIC FERRI - CYANIDE.	CARBOL FUSHSIN.
3.	MID GUT.	+++	+++	++

+ + + STRONGLY POSITIVE.

+ + MODERATELY POSITIVE.

+ MILD POSITIVE.

CHAPTER - 4

DISCUSSION.

Results and conclusin of the present study on changes occurring because of aging, are based on the results and observations performed on Gut of Male and Female Fishes, channa punctatus . However special attention was focused on the first appearance, distriubtion pattern, morphological characterestic and accumulation of Lipofuscin pigments with age. Qualitative and quantitative investigations were also made in different tissues at different age levels.

For intra-species comparisons fishes were divided into three progressive age groups (i.e.) premature, mature and post mature age groups, as four age groups were made by Brody (1960) for humans, two age groups by sharma (1967) for Ophiocephalus striatus , six age groups by Few & Getty (1967) for dogs and hogs, four age groups by Samorajski, Ordy & Reimer (1968) for mice, five age groups by Nanda & Getty (1971) for pige, six age groups by Vyas and Nanda (1981) for goat, four age groups by Goyal (1982) for mouse and five age groups were made by Gupta (1989) for Insects. and three age groups by Agarwal (1995) for channa punctatus .

For the histochemical comparsons, the representative tissues from the animals at different age levels were fixed in 10% neutral formaline solution for 3-4 days. Similarly tissues were fixed by Strehleret al (1959) in 20% formaline for 2 days and in 10% neutral formaline by Vyas & Nanda (1981) and in 10% neutral formaline by Gupta (1985).

Parrafin sections were cut at 6 u form Gut as section were cut at 10 by Brody (1960) from cerebral cortex, at 10-15 day Nandy (1968) from different parts of CNS, at 8 by Nanda & Getty (1971) from brain , at 4 by Goyal

(1981) from humand myocardium and sections were cut as 6 by Gupta (1983) from heart of Dysdercus similis .

In the present study sections were stained with Nile Blue a, ferric ferricyanide and carbol fuchsin method for microscopie examinations Similarly Nile blue A, Sudan black B, ferric ferricyanide and carbol fuchsin stains were used by Nanda (1971), Nile blue A and carbol fuchsin by Gupta (1985). The stained sections were observed under light microscope for lilposfuscin pigment granules. Similar observations under light and elctron microscope were used by samorajski, Ordy & Reimer (1968) and light microscope was used by Gupta et al ; (1989) and Agarwal (1995).

Results revealed that in the yougest age group one (pre-mature), No pigment deposition within the cells of gut was observed. Examinations of the present study the presence of Lipofuscin at early stages of age are fully convincing and are in aggrement with earlier findings of various investigators in different Organism. Jayne (1950) also reported similar observations in new born human heart. Further Brody (1960) found that the cells of Neurones of a new born Baby were free of Lipofuscin pigments. Lipofuscin pigment was not demonstrated in heart, etc of Dysdercus up to the age of 5 days, (Gupta et al) & Lipofuscin, pigment was also not observed in brain & heart of premature channa punctatus. (Agarwal 1995.)

Similarly Lipofuscin pigment was not detected in neurones of senile guinea pigs in first two months of age (1968). Brizzee et al, (1969, 1974) also failed to reveal the presence of lipofuscin in very young rat brain and in rhesus monkey brain until the age of three months. According to Strehler et al , (1959) no pigment was demonstrated in the heart of human beings below ten

years of age, Complete absence of lipofuscin pigments was demonstrated in the heart of child upto the age of six years (Hendley et al , 1963) . Munnell & Getty 1968 also found that there was no pigment in the heart of dog below six months of age.

Absence of Lipofuscin pigment in young specimens has also been describe by a number of investigators and its occurrence in the first age group has also been confirmed by several workers. Sohal 1971 has also reported that was no sign of lipofuscin pigments in the heart of house Fly upto the age of seven days. whereas Sohal & Sharma (1972) studied the brain of Hould Fly and did not find Lipofuscin pigment at the age of three days. Goyal (1981) Studied human heart and found no pigment granules in a person below nine years of age. Similarly Horn et al.(1981) reported the absense of pigment in addinal gland upto three months old rats.

The result of the present study regarding the presence of Lipofuscin at early stages of age are fully convincing and are in agreement with earlier findings of the various investigators in different organism. It has a great interest to examine the first appearance of lipofuscin pigment ranules. In the present investigation the first appearance of Lipofuscin pigment, occurred in gut cells of early mature fish (i.e.) gegning of the second age group the findings of the present study are in agreement with those of Sharma (1967), who observed first appearance and comparatively very few pigments bodies in the neuroness of young animals than those of old ones. Few & Getty (1976) observed First appearance of Lipofuscin pigment in the nervous system at the age of Five Months in dog and at the age of six months in hog. (Gupta & Gupta 1983) described that Lipofuscin pigment in the mid gut of Male Dysdercus appeard clearly in second age group.

Nanda & Getty (1971) observed that Lipofuscin pigment was present in all brain areas Studied in Pig but the first appearance of Lipofuscin pigment was reproted at the age of one years and two months in nuclleus Oliveris. Similarly Vyas & Nanda (1981) studied the nervous system in aging Goat and reported the first appearance of Lipofuscin. Nucleus motolius nervous - trochlearis and formatic reticularis at the age of one year and twelve days, in the nucleus tractus mesencephalici at the age of eight months and sixteen days in nucleus motorius nervous Occuiomotorii at the age of one years three months and eight days.

Goyal (1981) was of the opinion that the first appearance of Lipofuscin pigment at nine years of age in the left Ventricular myocardium. Similar obserbations were also made by whiteford & Getty (1966) in canine and porcine brain. According to them, hypoglossal nucleus in canine exibited evidance of Lipofuscin pigments at twenty five years of age where as in iferior olivary nuclleus, the accumulation of pigment appeared at the age of four years. Similarly in porcine brain hypoglosssal nucleus showed the first evidence of Lipofuscin accumulation at the age of three years and four months and in inferior olivary nucleus at the age of four years.

Munnell and Gutty (1968) have reported the first appearance of pigments in the human myocardium after 10 years. Munnell and Getty (1968) also compared the period of dog life with that of human life, they were of the opinion that 6 months to 1 year period of dogs life can be compared to the 10-20 years period of human . The periods are comparable Only when the sexual maturity taken in to consideration in both dog as well as human . In the present study age groups were also made on the basis of sexual maturity of fishes.

In the present investigation it was found that the first appearance of lipofuscin pigment is same in both organs of the fishes i.e. the midgut.

In the second age group it was observed that the process of pigments accumulation in midgut gradually increased. Staining with Nile blue A, carbol fuschin and ferric ferricyanide mehtods. Pigments were first observed as scattered granules in some cells of mid gut in the beginig of second age group. These findings may be corelated with the results or Brizzee et al (1974) who observed scattered granules in only some of the neurons of the inferior olive of primate at 6 months of age.

Cells of the midgut of early mature fishes were found to contain few lipofuscin pigment granules distributed in the cytoplasm near the nucler. This is in agreement with the findings of Few & Getty (1967) in dog and hog. Goyal (1982) also reported that nerve cells of mouse 5 months of age contained very few pigments. The pigments apperas to increase linearly nearly with age . Such linearity between pigment granules and age was also observed by Munnell and Getty (1968) in heart of aging canine Gupta (1984) observed linear accumulation of lipofuscin pigments in midgut of Dysdercus simils , and Patro et al (1992) also observed linear increase of lipofuscin accumulation in myocardial cells of albino rats.

With different stains lipofuscin was frequently observed in midgut cells. Lipofuscin pigments stained by Nile blue A method appeard as dark blue green granules. Similar observations were made by Sharma (1967) who reproted that lipofuscin pigments appeared as dark brown granules in fishes, hemiducylus and natrix Similarly Reichel et al (1968) observed that lipofuscin pigments appeard as yellow orange granules in ordents brain. Whiteford and Getty

(1966) observed lipofuscin pigments as blue green granules in the neurones of Canine and procine brain. Nandy (1971) also observed lipofuscin pigments as green yellow granules in younger mice.

The morphological characteristics of lipofuscin pigments in the mid gut were found to be very similar in the mature age group. Similar observations were made by Hess (1955) in neurones of man and small laboratory animals. Miquel (1971) was of the opinion that lipofuscin pigment granules in insects were similar in colour and size to the mammalian lipofuscin pigments and other common characterstics of mammalion and insects aging seems to be the accumulation of age pigments. The fine structure of lipofuscin granules in early age was described by Malkoff and strechler (1963) in human heart. Chirstensen (1965) and Frank & Christensen (1968) in guinea pig interstitial cells of leydig and Fawcett & Bargos (1960) in the interstitial cells of human testes.

In the fishes of mature age group pigment granules were observed rounded in shape and homogeneous in structure . The lipofuscin pigments appeared as a single granule scattered irregularly throughout the cytoplasm. These morphological findings are in agreement with the findings of Totaro and Pisanti (1980 a) who studied small rounded and homogeneous and scattered lipofuscin pigment granules in elctric lobes of young torpedo.

In the gut cells of fishes ranging in mature age group. the pigmentation usually started as tiny granules diffusely distributed in the cytoplasm. Similar observations were made by Nandy (1968, 1971) who reported diffusely distributed granules in the neurones of guinea pig at the age of 6 months Totaro and Pisanti (1979) the pigment granules appeared as tiny and diffusely distributed in newdorn torpedo.

In the present study it was observed that as age increased a moderate increase in the number of pigmented cells and pigment granules percells and maximum concentration of lipofuscin pigment was observed in the heart cells. Similar observations were made by Donato et al (1979) in housefly. Goyal (1981) also reported that the number of pigmented cell and pigment granules had increased in human myocardium and continued to increase in number and started accumulating gradually with age. According to Brody (1960) as one proceeds thorugh adult age group there is sharp decrease in the number of cells free from pigments and an increse in the number of cell containig piemgnts in human cerebral cortex. White Chu, (1954) found that n the human spinal cord the amount of pigments increases with age of the individuals and Wilcox (1959) after examination of the cranial nerves in guinea pigs, also stated that the accumulation of lipofuscin is corelated with aging.

In present investigation a greater number of dark granules become prominent and appeared to be larger in size in the mid gut . This may be corelated with the findings of Few & Getty, (1967), who reported that before function, the initial pigment bodies appeared to enlarge as their substructure become denser in nurons to hogs and dogs. Totarp and pisanti (1980 b) reported that in younger animals, the granules are quite small, on the other hand, pigment was clearly seen n neurones of adult torpedo, hase characteristics seems to evolve with age.

In mid gut cells of mature fishes the pigment granules lie scattered in the cytoplasm, but occasionally they show a tendency to aggregate, therefore few pigment granules were observed clue close to each other in late mature fishes. Similar fincings were made by Sharma (1967) in fishes and Goyal (1981)

in man. Nanda and Getty (1971) demonstrated that nucleus oliverls inferior nucleus olivaris of aging pig showed a tendency for lipofuscin accumulation at the age of 3 years and 9 months.

In the present study although exact quantitative evalulation was not parctical results of attempts to corelate the amount of pigments occurring in the neurones adn cardiac muscles with age indicate that in general pigmentation increases with age with respect to both the number of cells affected and concentration per cell. It is perticular interest to estimate the percentage of intracellular volume which is occupied by pigments. The percenatage of intracellular volume which is occupied by pigments. In present investigation the pigmented cell was observed approximately 40-60% from heart and 30-50% from brain cells of mature fishes. Similar observation were made by Nanda & Getty (1971) in aging pig, they reported that at the age of 3 years and 9 months nucleus olivaris inferior cells were pigmented 40-50% of from nucleus hypoglossus 60-70% and dorsal motor nucleus of vagus about 35-40% at the same age. Samorajski et al (1968) reported that 55% of dorsal ganglion cells and 47% of purkinge cells in aging mice were pigmented at 8 moths of age. According to whitford and Getty (1966) cochiear nuclel contained 40-50% of pigmented cells at 7-8 years of age where as 12-34% of mesencer nallcnucleus of the trigerminal nerve cells were pigmented at the age of 7-8 years respectively.

In the present study it was also observed that the rate of pigments accumulation is also affected by environmental factors. Data indicates that the rate of pigments accumulation is comparatively low in cultured habilat than that of natural habitat. Similar observations were also made by papatranges and

Lyman (1982) in mesocricetus (Hamster) They indicate that the rate of lilpofuscn accumulation in the hbernators is slower than that in the nonhivernators.

The thrid age group includes postmature fishes. the observations of mid gut cells of post mature fishes evealed variations in size and shape.

This may be correalted with the findings of Hassan and Gless (1973) who reported that pigment granules revealed varations in shape and size and electron density with increasing in age. In the cells of heart and brain of post mature fishes it was observed that two or morr pigment bodies fused in several instances to form larger pigment granules . Similar observations were mad by Patro et al (1992). who reported that the size of the pigment bodies tended to increase with increased age and deposition of pigments according to them, in the myocardial cells of young animals the pigment bodies were mostly in the form of granules measuring 1.25 ml diameter and those found in the cells of the adult and senile animals had a greater tendency towards the formation of loose aggregates and duplexes and the average size of the pigments particultes increased to 3.95 - 5.43m in the adult and senile myocardial cells respectively.

In the present investigation as the fish advanced in age the pigment granules appeared dense and heterogeneous in structure . Similar observations were made by Few and Getty (1967) in hogs and dogs. Brizzee (1969) also reported that the predominantly scattered fluorescent bodies were found in young and adult, and the tendency of these mateial to accumlate in clusters was reported in perikaryon in ages rat.

In addition, the dense bodies were more heterogeneous and become more irregular in the older animals, there was tendency for these pigments to collect in to groups througout the cytoplasm. The intracellular pigments

distribution is in the agreement with the findings of Muhlmann (1910) who noted that the homogeneous distribution of pigment granules in ganglion cells of the guinea pigs and man was gradually lost with increasing age, gathering in clusters mass which continued to increase in size. Similar observatios were also made by Samorajski et al (1964) in neurones of aldult human beings.

In the post mature fishes the majority of the midgut cells contained pigment granules. However greater number of these pigmented cells contain scattered pigments and only a relatively small number showed clumped pigments in the cytoplasm. This is in agreement with the findings of Brody (1960) who reported that cortical cells in human brain contain scatlered pigments as well as clumped pigments in the cytoplasm. The number of cells containing pigments increases with increasing age Goyal (1981) is of the opinion that pigment bodies appeared in accumlated and concentrated masses in human myocardium in the fifth decade. Munnell & Getty (1968) also revealed te clumping of lipofuscin granules in dog with the advancement of age .

It was clearly Observed in the midgut cells that the size complexity and intracellular distribution of these pigments was variable to some extent among different cells even of the same section . In the gut most of the granules occupied the entire cytoplasmic area, white few of them were deposited at eh periphery of cells. Similar observations were made by Jayne (1950) and Hasan & Gless (1973), who revealed variatoins in size, shape and distribution of pigment bodies among different cells even of the same section. Samorajski et al (1968) reported that dark pigment bodies appeared to be larger in size and were often concentrated within the peripheral portion of perikaryon in aging mice.

In present investigation all the four categories of cells were found in the cells of midgut of post mature fishes et al cells without pigment, cells with few pigments cells containing homogeneous pigments and cells containing heterogeneous or clusters of pigments granules distributed throughout the cytoplasm. Similar observation were made by Nandy (1971) who reported the following three categories of cells in aging mice i.e. cells with no pigment , cells with few diffuse pigments and cells showing heavy (clumpy) pigmentation. Similary Gupta (1985) also reported all categories of cells in the heart cells of Dysdercus simils during the investigation of fourth age group.

In the present study the pigment granules and pigmented cells increased inearly with age similar observations were made by Streheler el al (1959) who reported that lipofuscin granules increased lineraly in cardiac. Musscles fibres throughout life. Similar corelation of increased accumlation of fluroescence with age have been shown by shecal & Tappal (1974) in aging

DROSOPHILA.

In present investigation , heavy deposition of pigments observed in heart where as brain cells show comparatively low deposition of lipofuscin granules. These results are in agreement with be findings of Brody (1960) who reported the highest percantage of pigmented cells in the precentral gyrus and the lowest percantage in striate cortex of old human brain. While Donato and Sohal (1978) noted that the greater number of fluroescent granules appeared within the epithelial cells of the midgut where as heart and malpighian tubule contained less flurorscent granlules in male housefilies ranging in age from 15 to 25 days but, papafranges el al (1982) have also reported more pigment bodies in

in the brain tissue than in the heart tissues of hamsters.

In present study maximum percentage of pigmented cells was observed in different tissues of post mature fishes. The percentage of pigmented cells in heart was 60-85% and in brain 52-75% cells were pigmented . The highest percantage of pigmented cells was noted in heart. Thses results are in agreement with the findings of Nanda and Getty (1971) in the nervous system of aging pig. According to them 80-90% of purkinje cells of cerbellar cortex and about 75-85% of neurones were pigmented by 10 years of age. Samorajski el al (1968) described that 85% of dorsal ganglion cells and 98% of purkinje cells of agng mice were pigmented by 30 months of age.

The present observations indicate that lipofuscin pigment was present in all the tissues studied. The amount of pigments as well as percentage of pigmented cells increased with age. The microphotographs show that the pigmentation in mid gut increases progressively with age. The linear relationship of accumulation of lipoffuscin with age has been established and statistically signigicant positive corelations was found. Strehler el al (1969) in man and Munnell and Getty (1968) in canine also ablished similar relationship.

In the present investigation Nile blue A, carbol fuschin and ferric ferricyanide mehtods were used for histochemical studies of lipofuscin pigments in mid gut of male & female of channa punctatus in different age groups.

It is very interesting to note that lipofuscin pigments in the cells of the mid gut in different are groups reacted strongly to lillie's alternative Nile blue A. method . This in agreement with the findings of Sharma (1967) who reported that pigment granuels in the nerve cells of Rana, Bufo, Uromastix and Natrix reacted strongly with Nile Blue A. Totaro et al (1981) were of the opinion

that lipofuscin pigments in CNS of merine teteostei Sargus s. and scorpeoena s had strong affinities with lillie's alternative Nile blue A method.

The lipofuscin pigments in the midgut exhibit variations in the staining properties in mature and post mature fishes as pigments in different tissues of mature fish reacted moderately to Schomorl's ferric ferrcyanide method, whereas old tissues had strong affinities to this stain. These findings are similar to those of Nandy (1971) in neurones of young old mice . He reported that in younger animals lipofuscin pigments were more easily stained with sudan black B and PAS while on othr hand in olde ranimals , it was easily stined with Nile blue A and ferric ferriyanide mehtod.

Totaro et al (1981) also described that pigments had characteristically different staining behaviour in marine teteostei. According to them in central nervous system of Sugus a and Scorpoena s lipofuscin exhibited a moderate posivity in Schmorl's method whereas in Cons j lipofuscin reacted mildly Totaro el al (1981) also reported major variations in staining properties in myocardium of merine teleostei.

The lipofuscin in the midgut showed great variations inthe staining properties with carbol fuchsin. In the second age group the pigment granules in heart related moderately whereas in the brain exhibits a mild postivity while in, third age group both the tissues. reacted moderately. This is in agreement with findings of Nandy (1971) in nearones of young and old mice According to Sharma and Manocha (1977) the pigments showed characteristically different staining behaviour as one granule may be moderatelly or intensely positive to a particular stain and the other lying in tis vicinity may be negetive or partly negative and partly positive. Manocha & Sharma (1978) were also made similar

observations in spinal cord squirrel monkey.

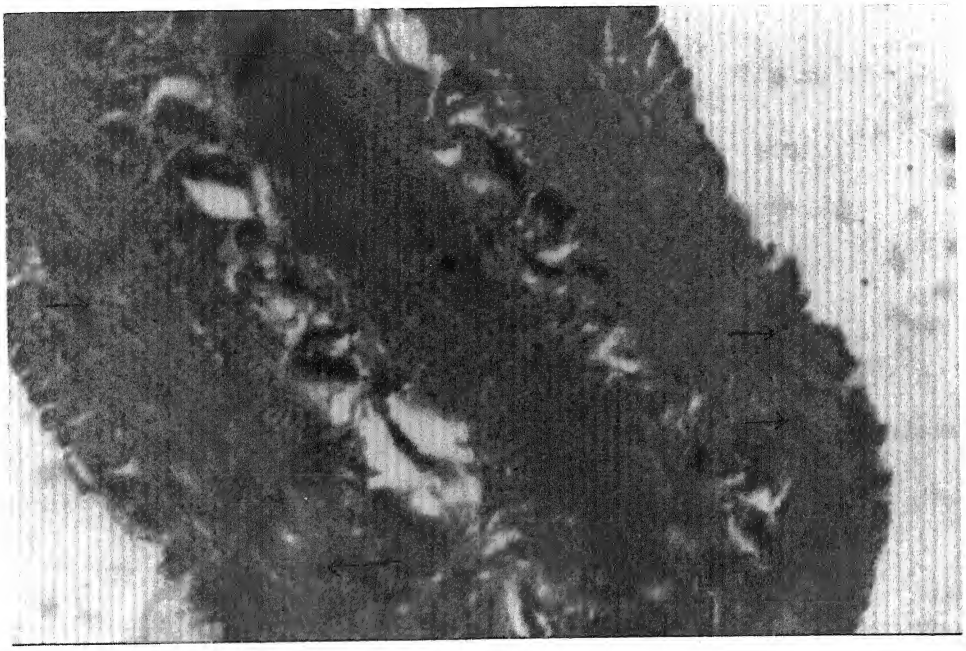
It is interest that in all the tissues of young fishes, lipofuscin pigments weremore easily stianed with Nile blue A than that of ferric ferricyanide and carbal fuchsin methods. On the other hand pigment granules in the mid gut in older fishes reacted moderatley with all the stains used. These varitations may be due to differences in the composition of lipofuscin pigments. The findings of Sharma (1967) in merine teleostel showed similar esults Gupta & Gupta (1985) reproted that in midgut of male Dysdercus in age group second, the pigment granules apeared in the cytoplasm cleared with Nile blue A than ferric ferricyanide they were of the opinion that pigment granules in older age reacted with both the stians. Strehler (1964) reported that a typical section stained with Sudan black B is essentially similar in appearance to the sectin stained with Nile blue sulphate.

It is evident form the present study that the affnities of lipofuscin with different stains varied cansiderably in different age groups. and these variations may be due to different composition of lipofuscin pigments at different age levles.

As such it is very clear from the findings of the present study that the accumulation of lipofuscin pigments gradually increased with age. The statisical analysis of data further cnfirms the fact the accumulation of lipofuscin is an age oriented.

Fig -1 Section of midgut from premature fish cells showing no pigment and rounded and centrally palced, nucles (arrows) appeared Nile Blue A Stain x 450.

Fig -2 Section of midgut from premature fish cells without lipofuscin pigments. Nucleus (arrows) centrally placed carbol fuchsin stain x 450.



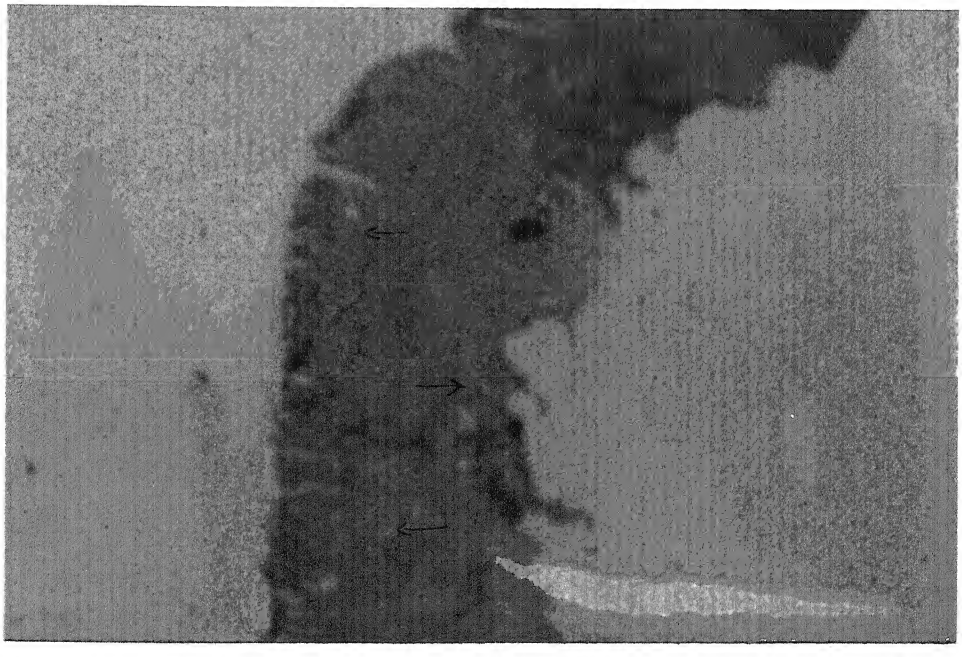


Fig -3 Section of midgut from premature fish cells centrally palced nuclei (arrows) but lipofuscin pigments did not appear. Ferric ferricyandie stain x 450.

Fig -4 Section of midgut from premature fish cells showing no pigmentation nucleus (arrows) has great affinity with stain. Nile blue A stain x 450.

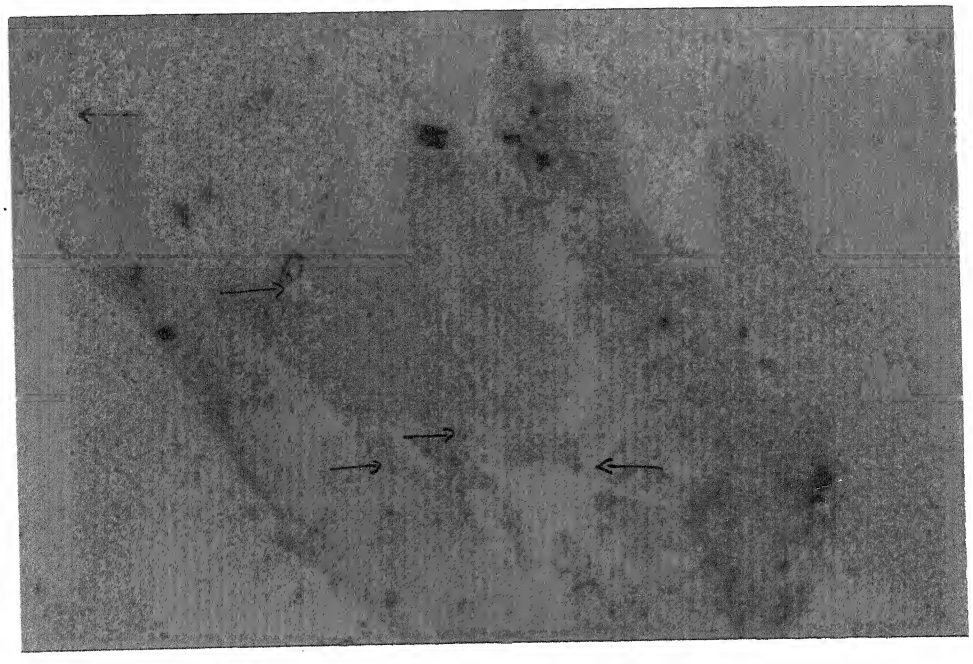
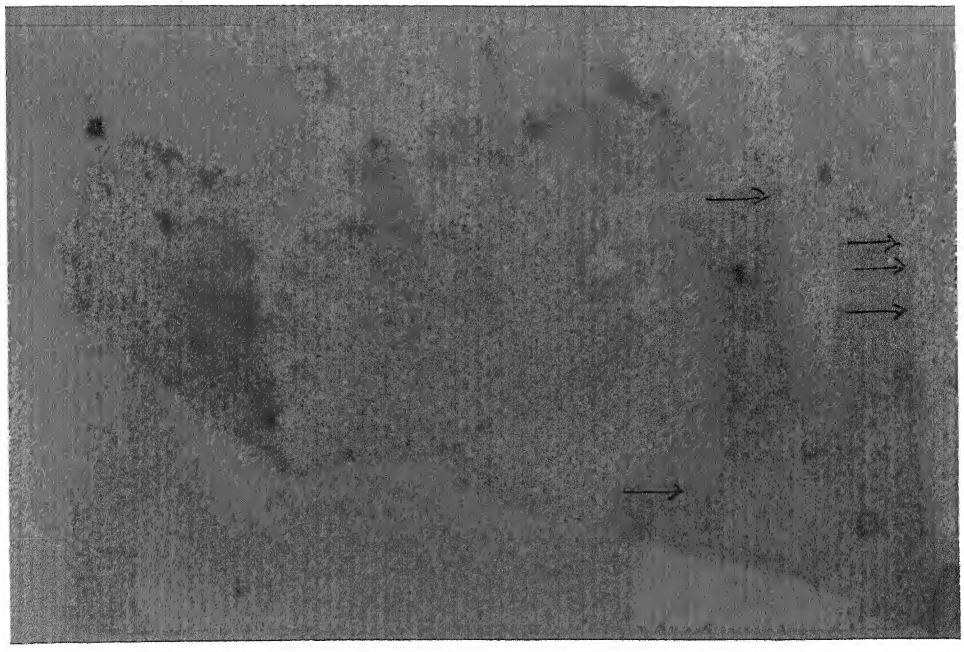


Fig -5 Section of midgut from premature cells showing no pigments. Nucleus (arrows) has great affinity with stain. Carbol fuchsin stain x 450.

Fig -6 Section of midgut from premature fish cells without lipofuscin pigments. Nucleus (arrows) Centrally placed and has a modrate affnities with stain, ferric ferricyanide stain x 300.



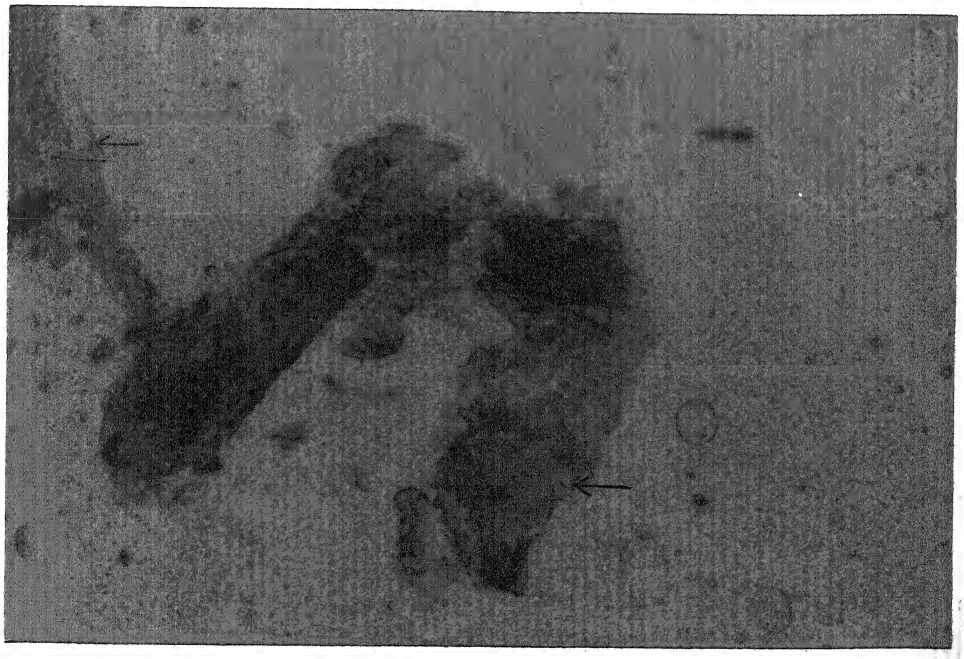
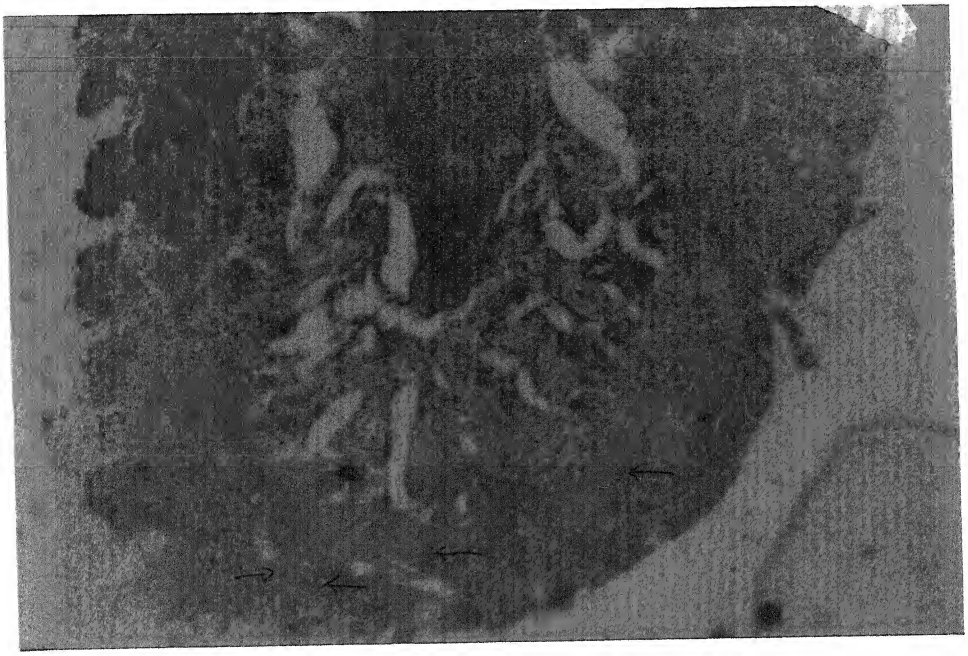


Fig -7 Section of midgut from early mature fish cells showing first or early appearance of lipofuscin pigment granules (arrows) carbol fuchsin stain x 450.

Fig -8 Section of midgut from early mature fish cells showing first appearance of lipofuscin pigment granules (arrows) Nile blue A Stain x 400.



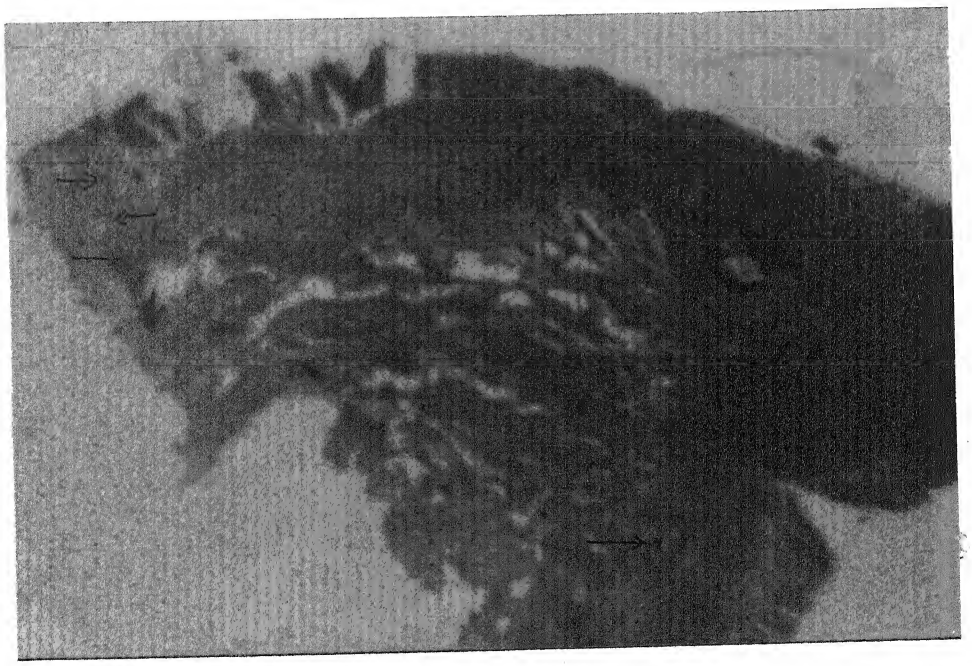


Fig -9 Section of midgut from early mature cells showing early appearance of lipofucin pigment granules (arrows) pigment appeard as tiny dot like and blue green in colour. Nile Blue A Stain x 450.

Fig -10. Section of midgut from early mature fish cells showing first appearance of lipofuscin pigment granules (arrows) ferric ferricyanide stain x 300.

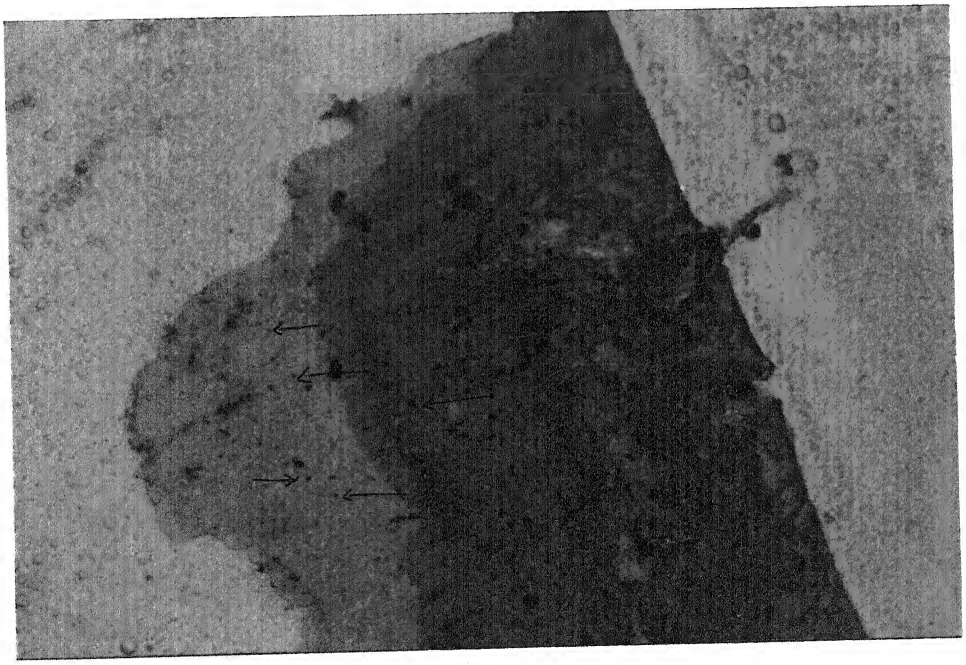
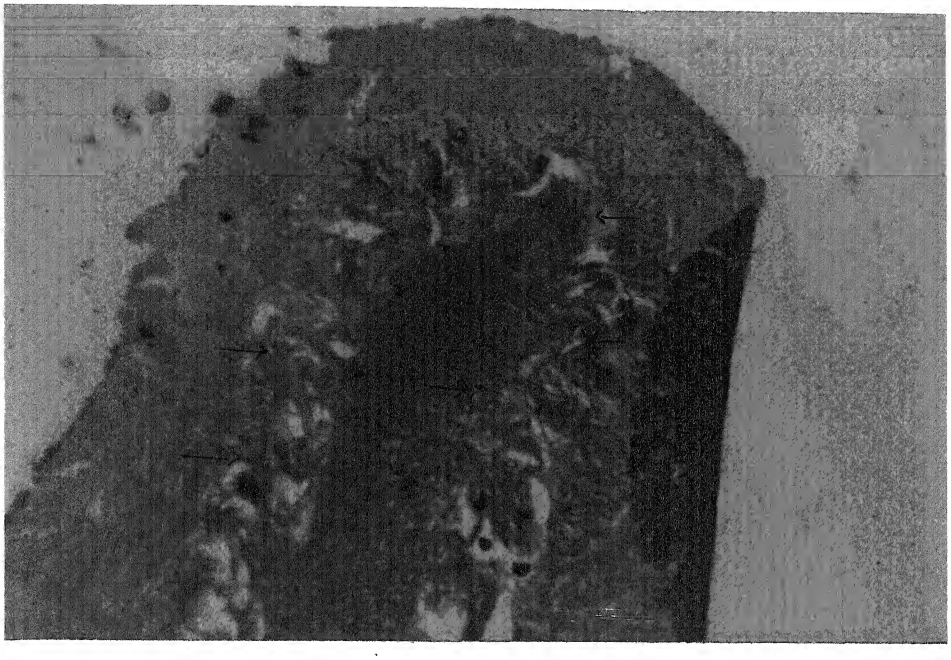


Fig -11 Section of midgut from mature fish cells showing increased accumulation of lipofuscin pigment granules (arrows), ferric ferrcyanide stain x 450.

Fig -12. Section of midgut from mature fish cells showing increased accumulation of dark blue green prominent pigment granules (arrows) carbol guchsin stain x 450.



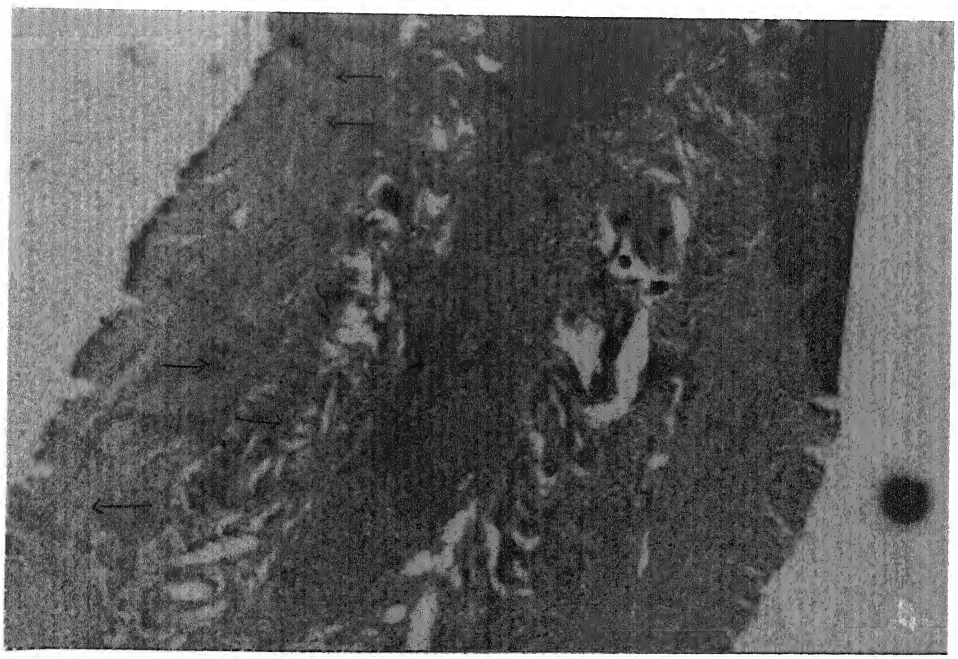
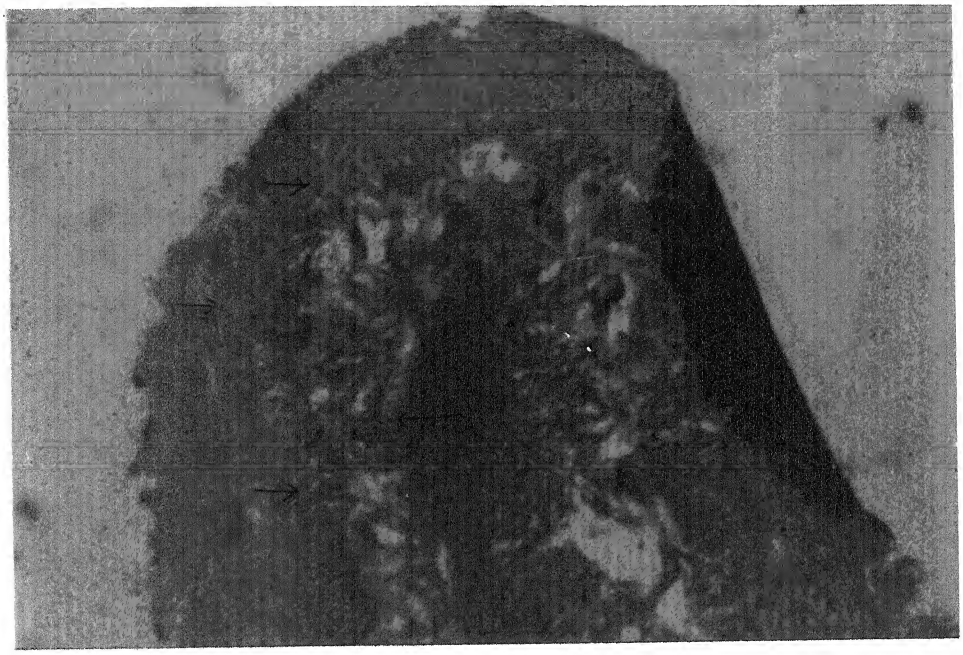


Fig -13 Section of midgut from mature cells showing moderate rounded and homogeneous pigment granules. (arrows) Scattered through out the cyotoplasm. Nile blue A Stain x 300.

Fig -14. Section of midgut from mature fish cells showing rounded and homogeneous pigment granules (arrows) distributed throughout the cytoplasm. Ferric Ferricymide Stain x 300.



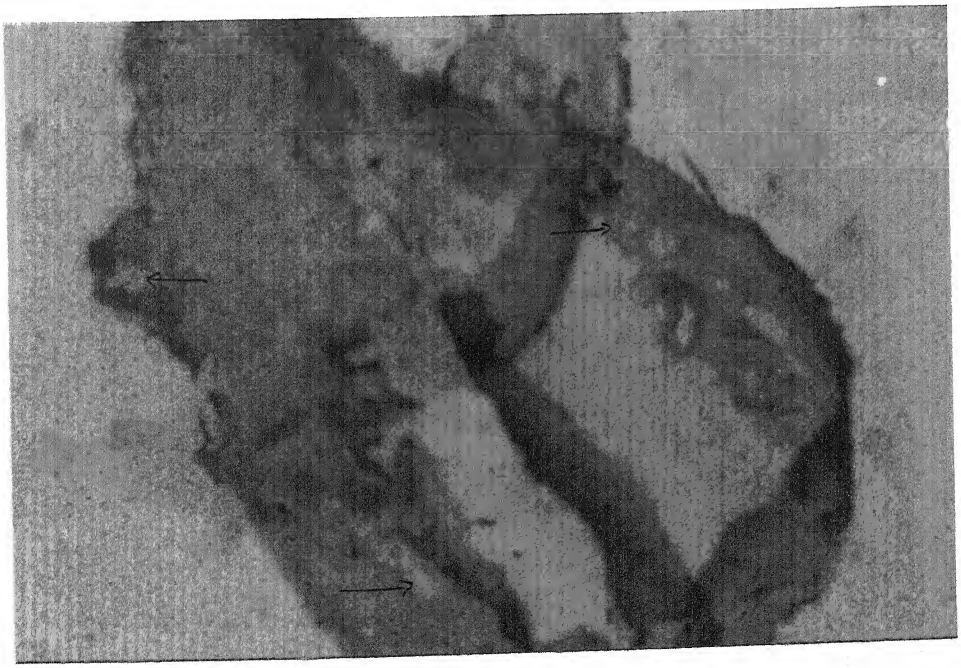
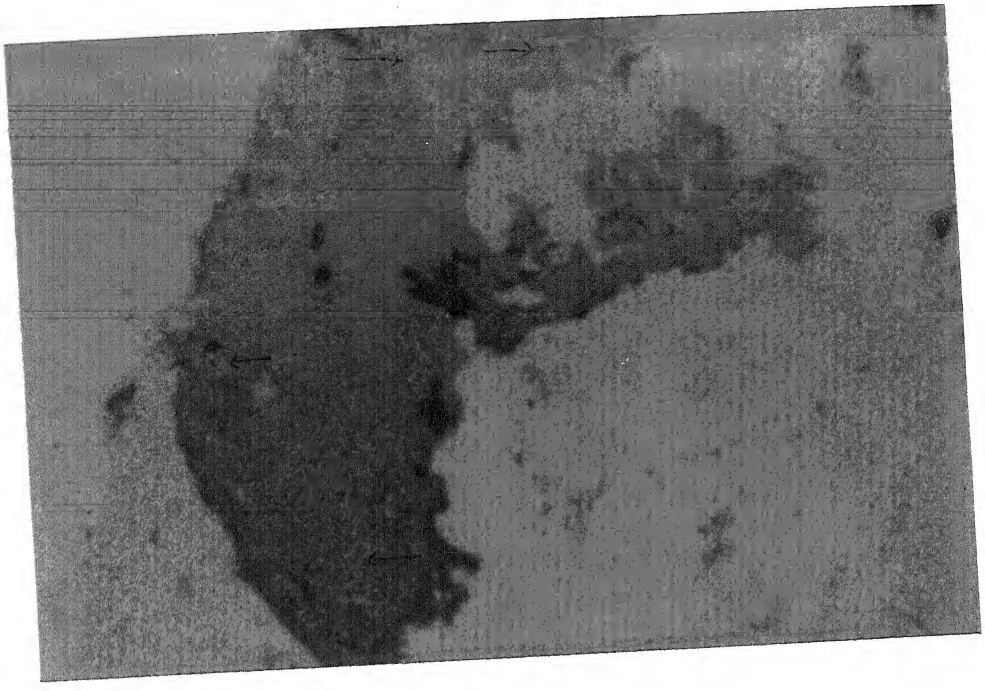


Fig -15 Section of midgut from mature cells showing rounded and homogeneous pigment granules (arrows) distributed thoughout the cytoplasm.
Ferric Ferricynide Stain x 450.

Fig -16. Section of midgut from mature fish cells showing moderate, rounded and homogeneous dense pigment granules (arrows) irregularly distributed through out the cytoplasm. Nile blue A x 450.



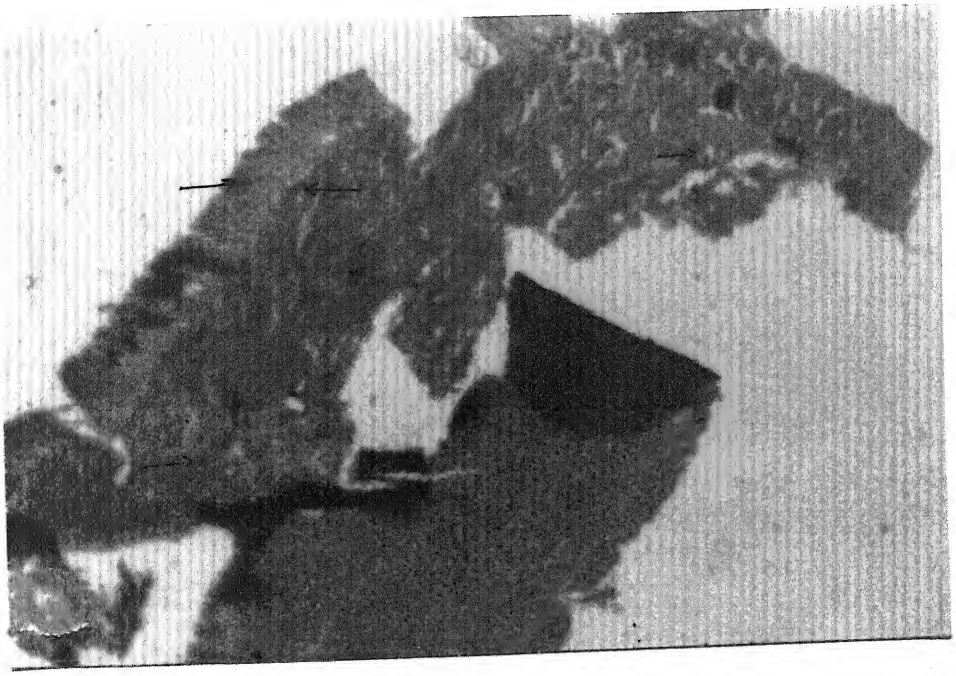
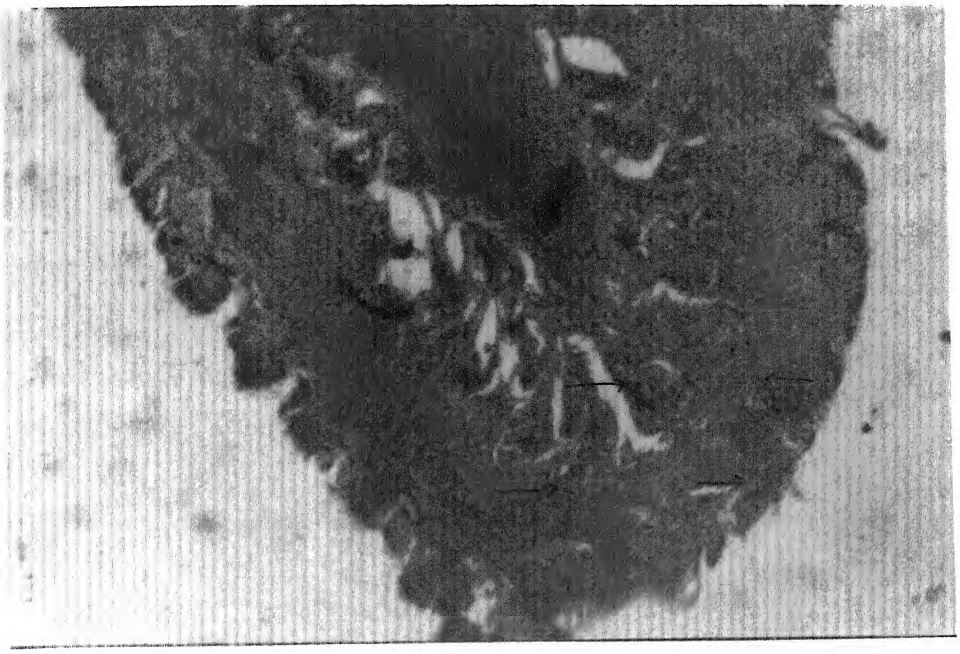


Fig -17 Section of midgut from late mature fish cells showing incresed accumulation of irregularly distributed lipofuscin pigment granules (arrows) Nile blue A Stain x 450.

Fig -18. Section of midgut from late mature fish cells showing dense and increased accumulation of lipofuscin pigment granules (arrows) with in the cytoplasm. Nile blue A Stain x 300.



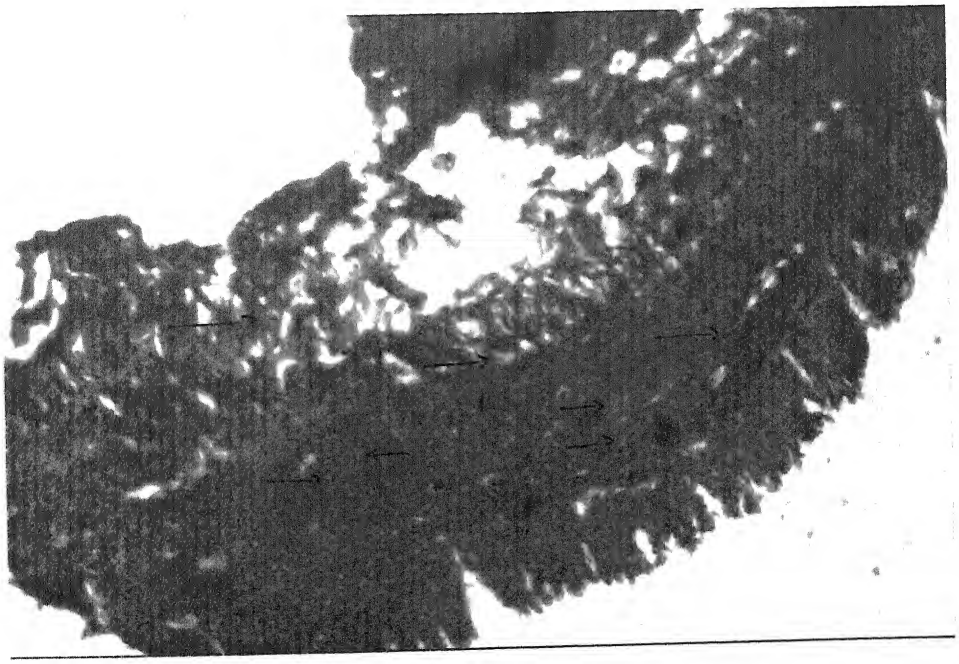
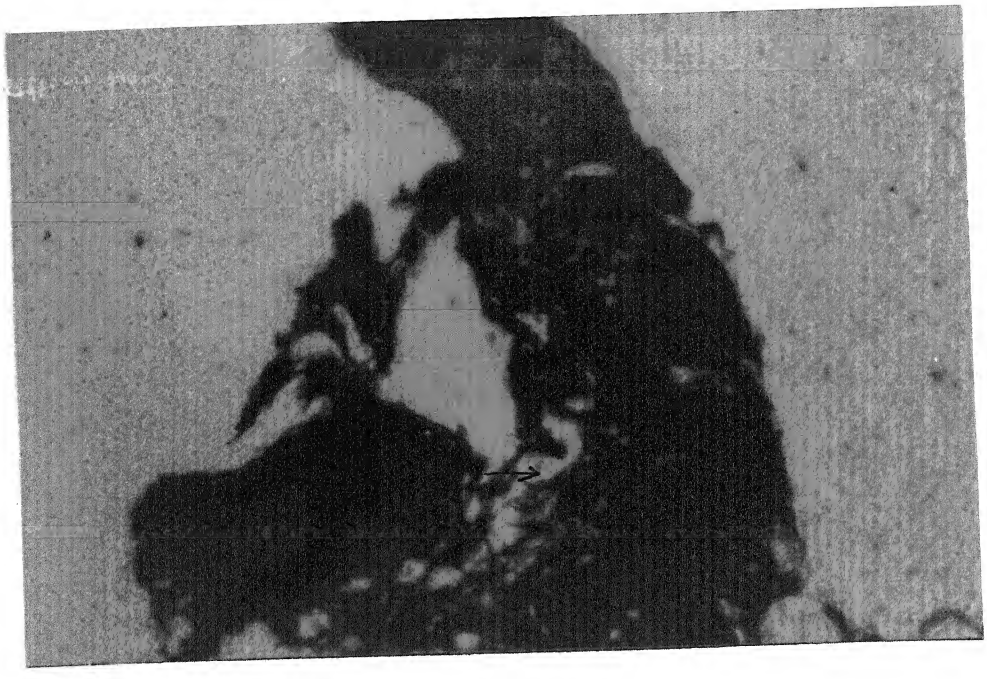


Fig -19 Section of midgut from mature fish cells showing tiny small lypofuscin ,pigment granules (arrows) Scattered thoughout the cytoplams. Nile blue A Stain x 300.

Fig -20. Section of midgut from late mature fish cells showing rounded and homogeneous lipofusin pigment granules (arrows) distriuted thoughout the cytoplasm. Carbol fuchsin stain x 300.



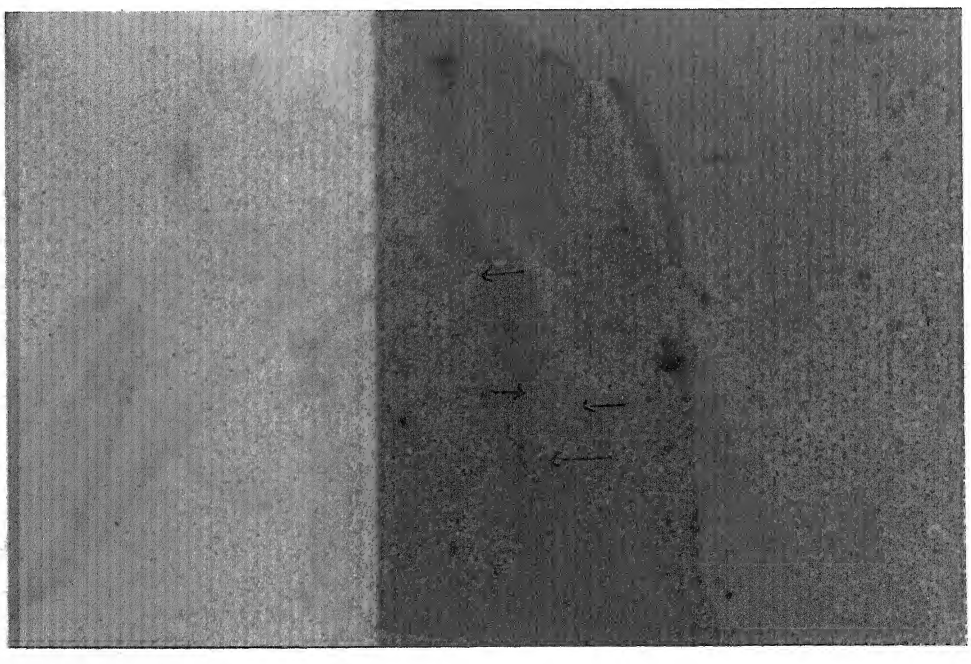
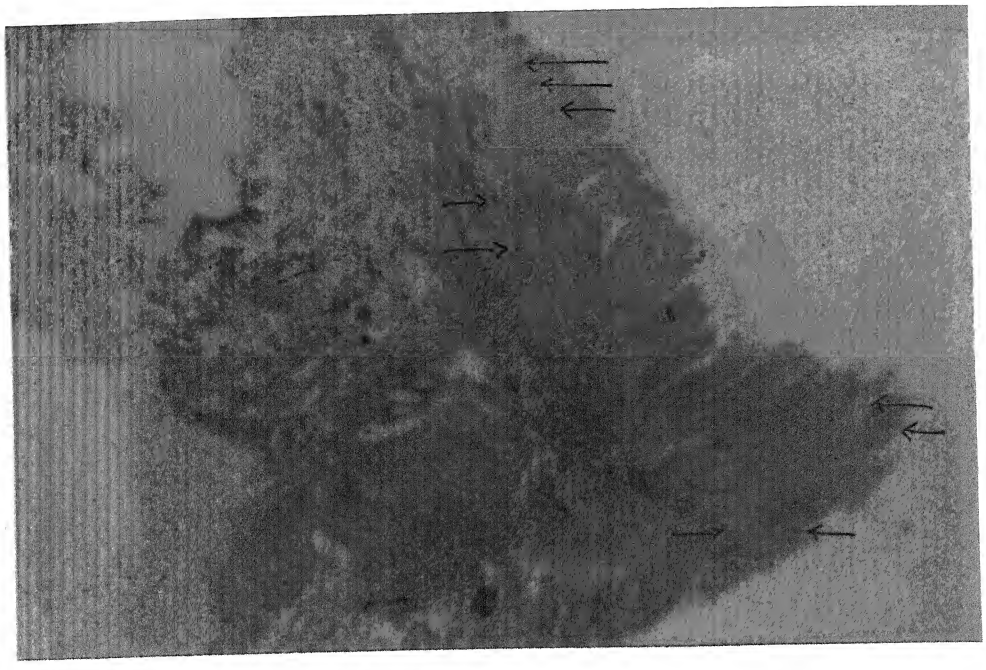


Fig -21 Section of midgut from late mature fish cells showing small rounded and homogeneous lipofusin pigment granules (arrows) distributed throughout the cytoplasm. Ferric Ferrcyanide Stain x 300.

Fig -22. Section of midgut from late mature fish cells showing dence and increased accumulation of lipofuscin pigment cytoplasm Carbol fushsin stain x 300.



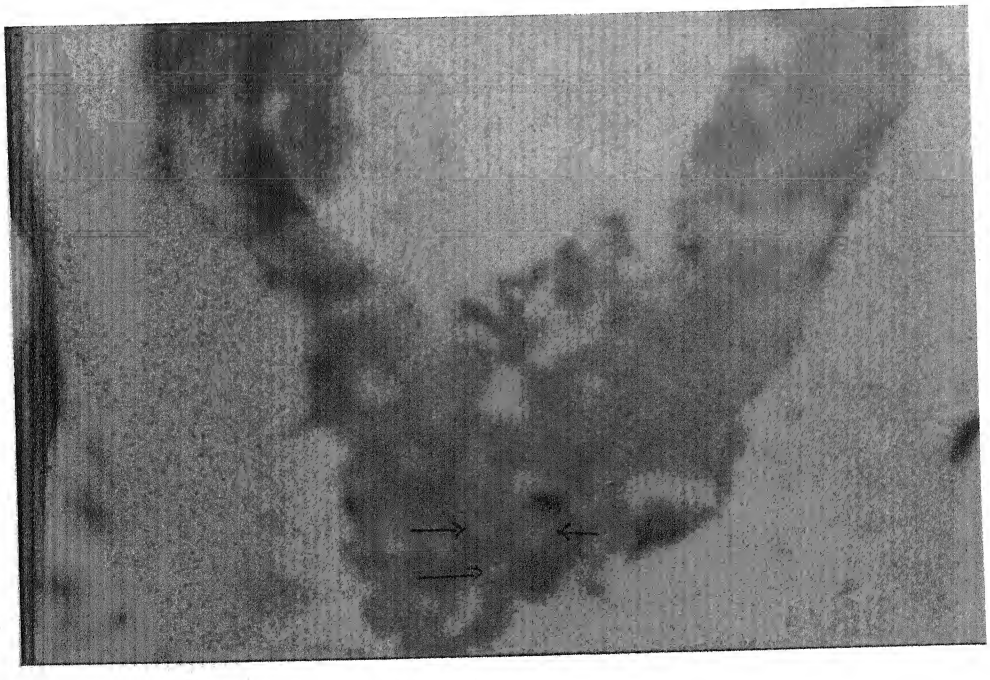
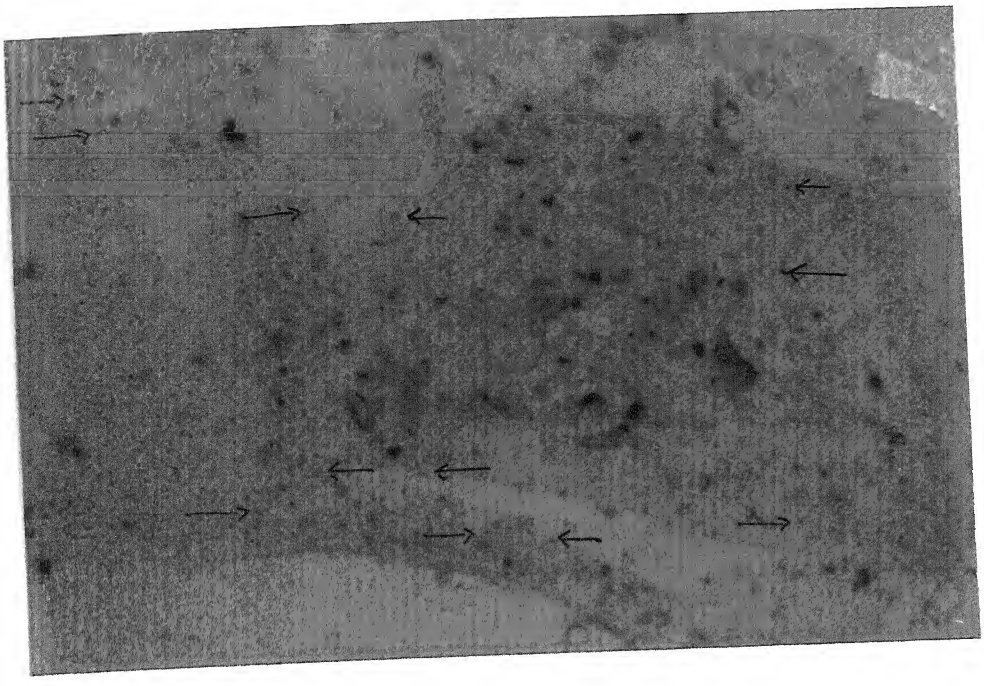


Fig -23 Section of midgut from post mature fish cells showing dense. clumped. hederogeneous, lipofuscin pigment granules (arrows) irregularly distributed thorughout the cytoplasm. Ferric Ferricyanide stain x 450.

Fig -24. Section of midgut from post mature fish. cells showing dense heterogeneous lipofuscin pigment granules (arrows) scattered thoughout the cytoplasm. Carbol fuchsin stain x 300.



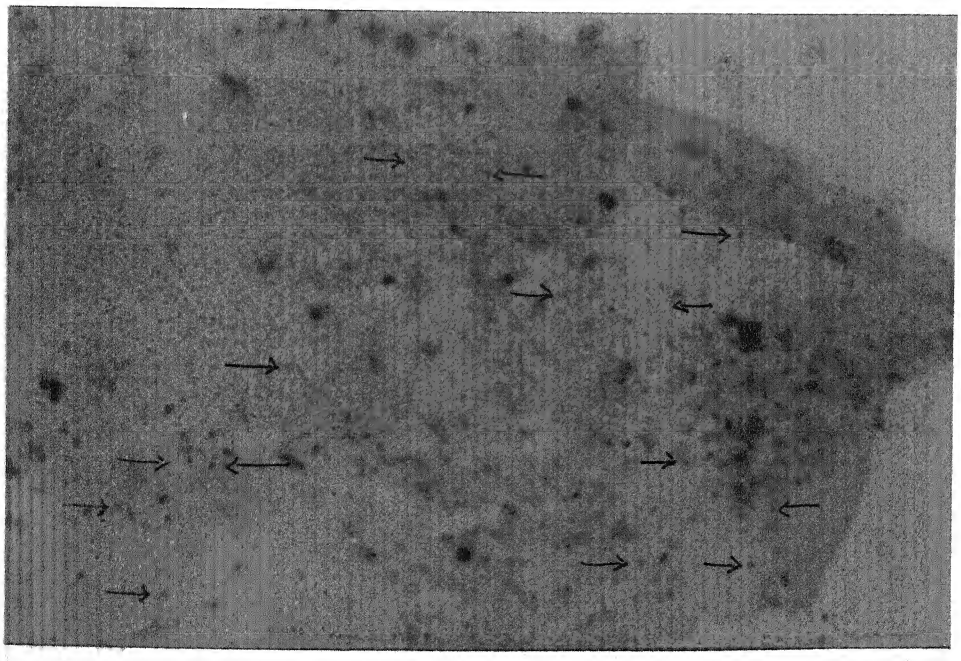
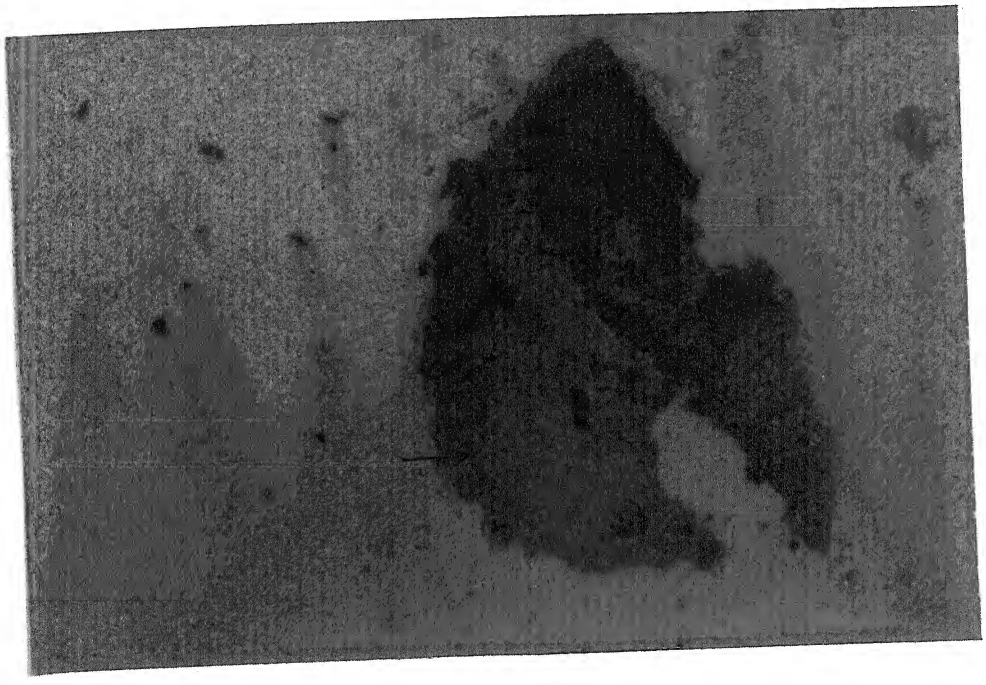


Fig -25. Section of midgut from post mature fish cells showing heavy depostition of cluped, hetergoeneous lipofuscin pigment granules (arrows) throughout the cytoplasm. Ferric Ferricyanide stain x 300.

Fig -26. Magenification of a portion of midgut from post mature fish, cells showing heterogeneous lipofuscin pigments granules (arrows) ferric ferricymide stain x 650.



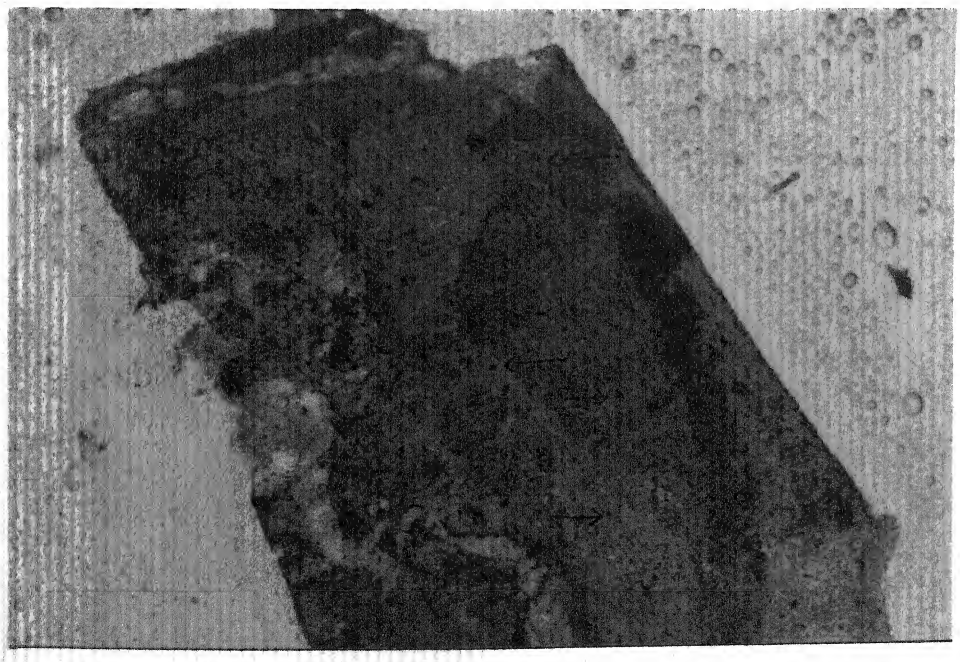
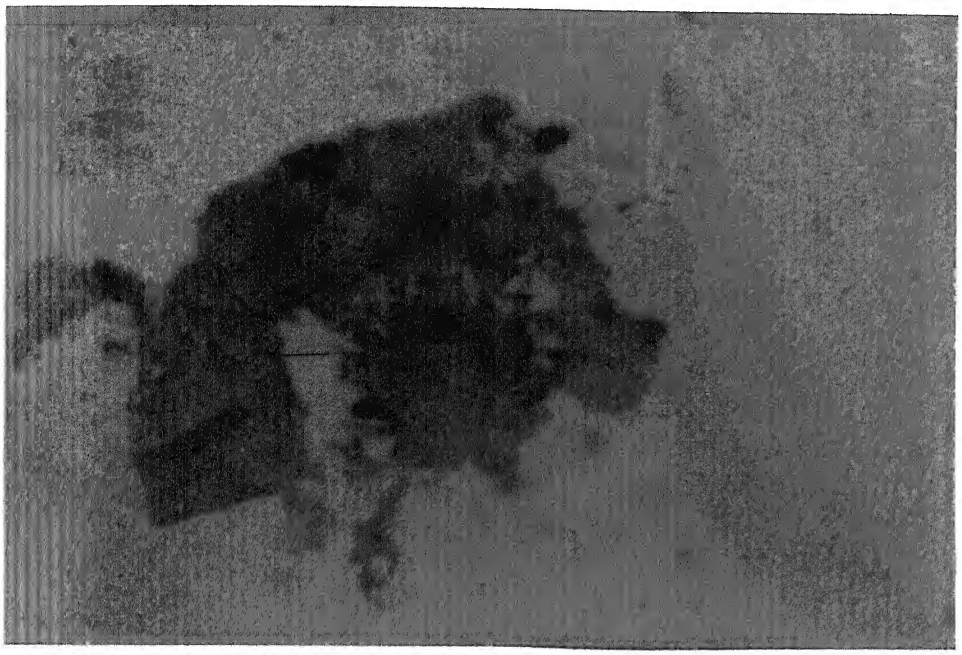


Fig -27. Magnification of a portion of midgut from post mature fish, cells showing heterogenenous lipofuscin pigment granules (arrows). Nile blue A Stain x 650.

Fig -28. Section of midgut post mature fish cells, showing agregated hetrogeneous pigment (arrows) irregularly scattered thoughout the cytoplasm.
Carbol fuchsin stain x 300.



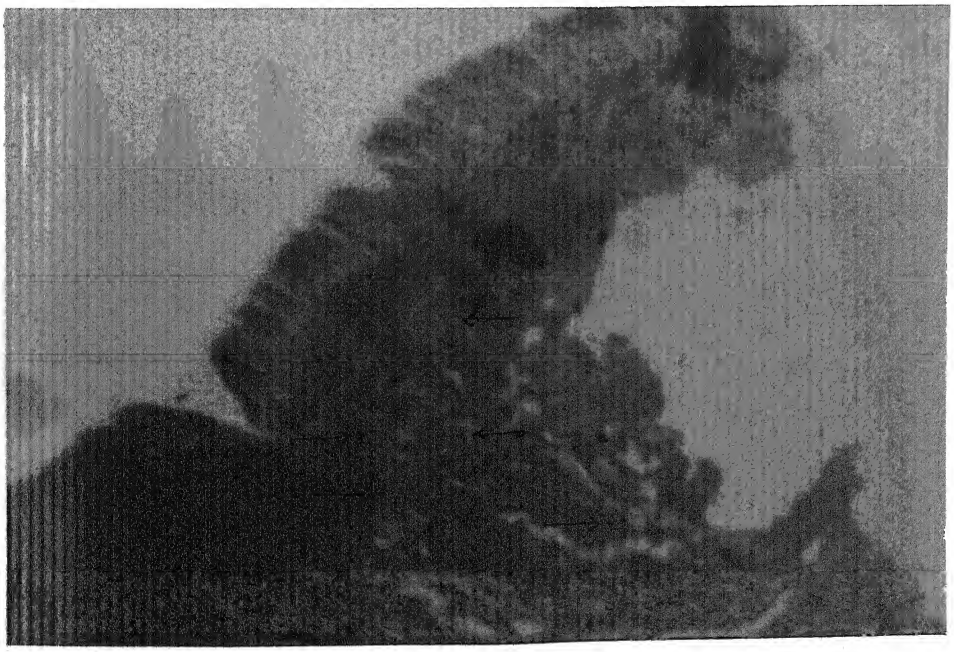
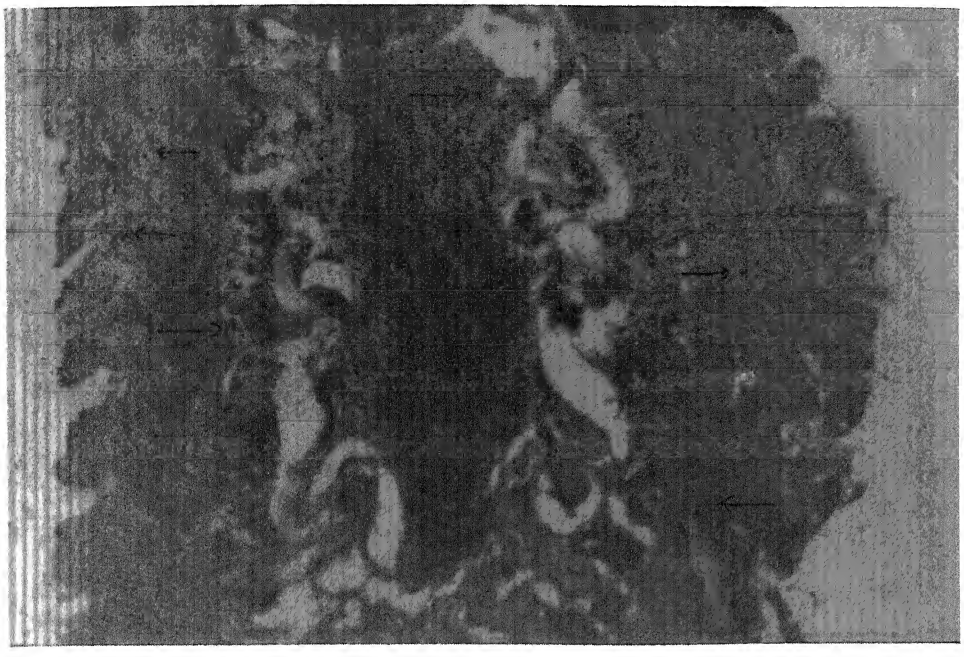
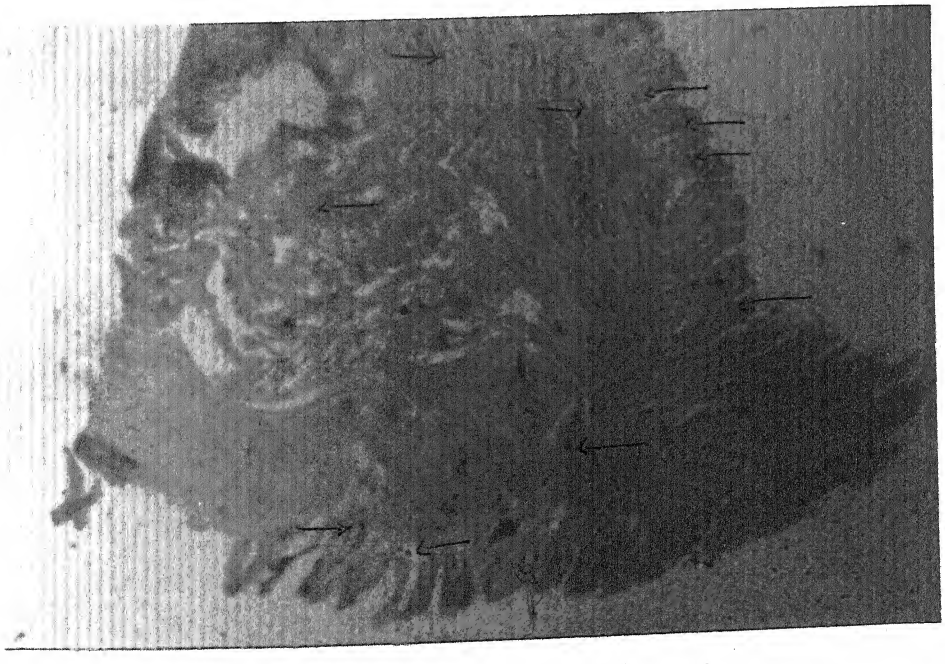


Fig -29. Section of midgut from post mature fish. Cells showing heavy depostion of heterogeneous lipofuscin pigment granules (arrows). Nile blue A Stain x 300.

Fig -30. Section of midgut post mature fish cells, showing heavy deposition of hetergoeneous lipofuscin pigments granules (arrows) carbol fuehsin stain x 450.





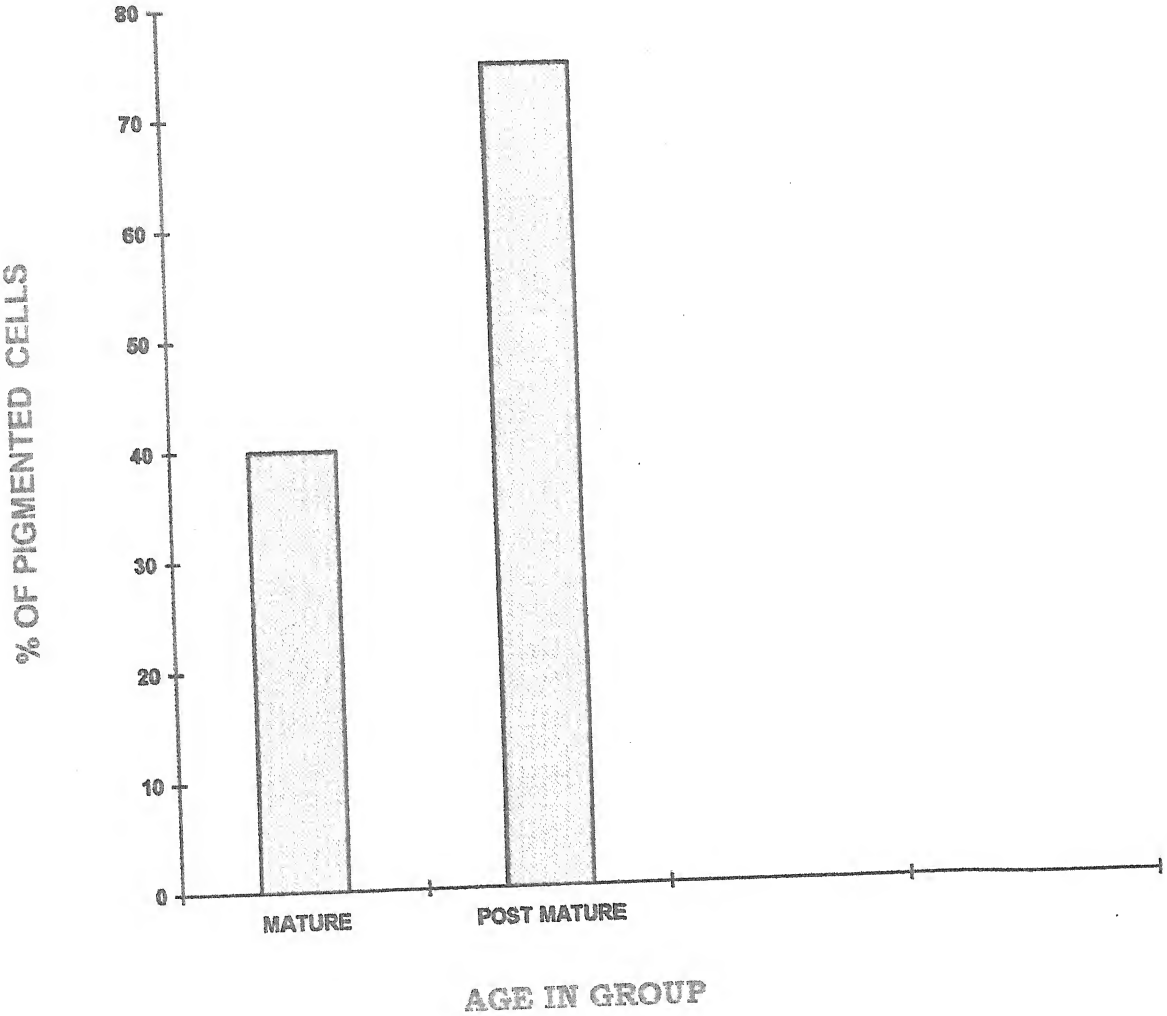


Fig -1 Precentage of pigmented cells in midgut of Channa Punctatus in different age groups.

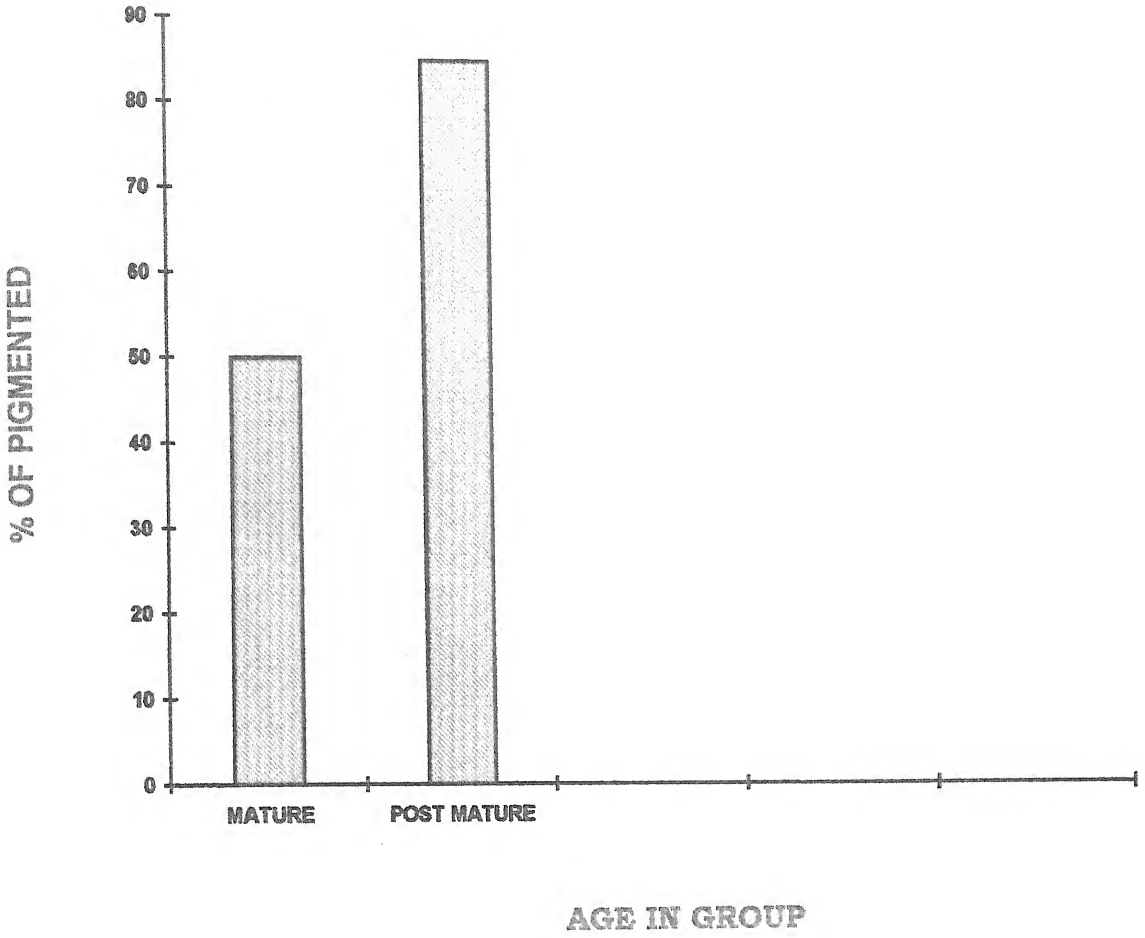


Fig -2 Precentage of pigmented cells in midgut of Channa Punctatus in different age groups.

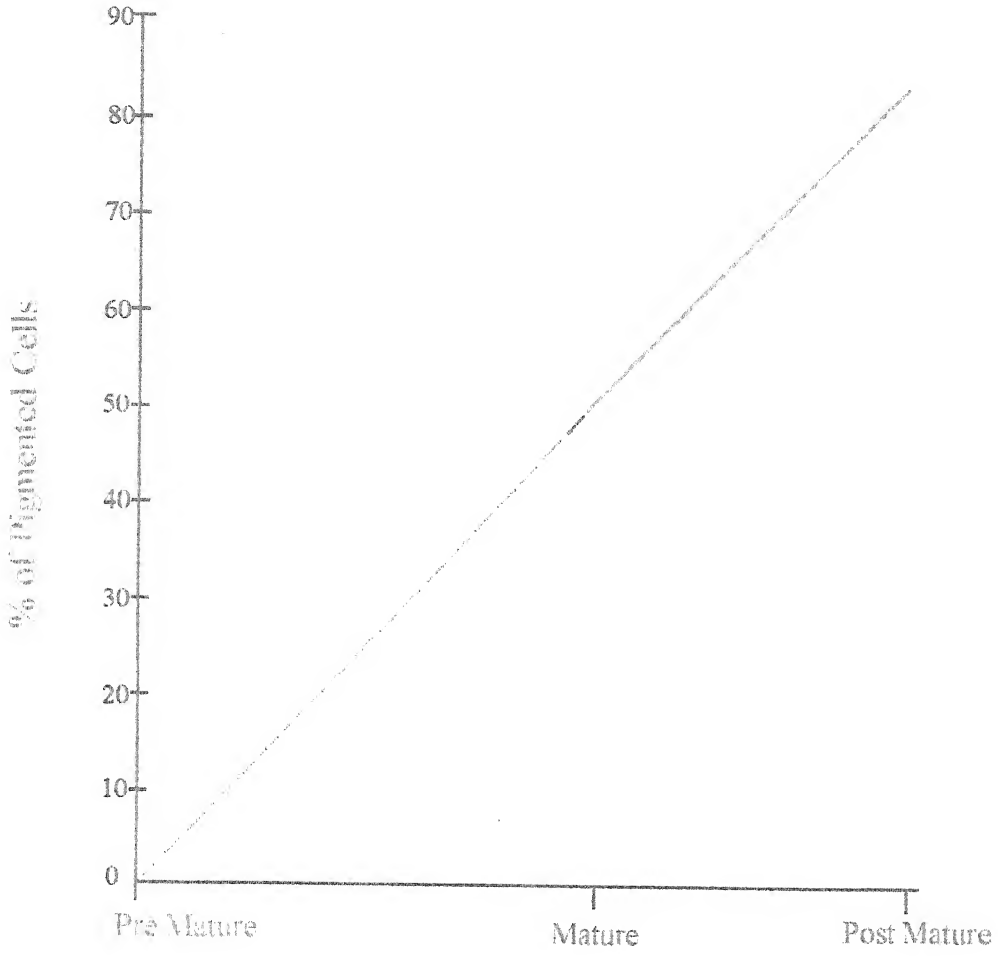


Fig -3 Precentage of pigmented cells in midgut of Channa Punctatus in different age groups.

CHAPTER - 5

SUMMARY

Objectives of the present investigations were to determine the first appearnce of lipofuscin pigments, its occurrence, the presence of pigmented cells per volume distribution morphological characteristics and staining properties of lipofuscin pigment in the midgut of Male & Female of Channa punctatus at various age groups. Qualitative and quantitative investigations were also made in tissue in different age groups. It was also observed that the time of the appearance of pigments and its subsquent increase varied in the organs at different age levels. the tissues i.e. midgut was examined in three progressive age groups. i.e. premature mature and post mature respectively.

Results of the present study revealed that the cells were found without lipofuscin pigments in mid gut of premature fishes. The lipofuscin pigments were first observed in cells of mid gut of early mature fish or at the beginning of second age group. An interesting finding of present investigation is that the pigment granules and pigmented cells increased propressively with age of fish. In the begning of the second age group the pigment granules were observed to be very few in number, homogenous instructure and irregularly distributed within the cells the mid gut . The pigments were also observed so abundant that they often occupied the entire.

The complete aggregation of pigment granules were observed in the third age group. As the fishes increased in age the granules anlarged and tended to form clusters. The pigment granules appeared to be larger in size and the intracytoplasmic location of pigment granules generally varied with age.

granules usually occupied the entire cytoplas nic area of the cells and few noted peripheral in position . Heterogeneous lipofuscin oigments were clearly obsended in this age group. All the four categroies of cells were observed in the cells of mid gut of fishes of third age group.

Results revealed that accumulation of lipofuscin pigment was found to increase upto third age group in all the tissues examind. As pigmentation propressed more and granules appeared to from clusters. In midgut mojority of cells contained clumped pigments instead of scattered granules in cytoplasm . The distribution of pigment was not unifrom in both the tissues i.e. most of pigment graules were found to scattered in the cytoplasm near the nuclei, while there were only few pigments deposited at the periphery. Different sizes and variable amounts of pigment granules recoreded in mid gut at different age levels in all age groups .

Result revealed that the pigmented cells increased progresivley in the tissue.

Histochemical observation of the mid gut of aging Channa punctatus revealed that the pigment granules showed characteristically different staining behaviour. These variations may be due to differneces in the composition.

of lipofuscin pigments. In the present investigation it was clearly noted that lipofuscin pigment granules in the midgut of mature and post mature fishes were easily stined Lillt;s Nile blue A method. thus showing a strong positivily to the pigment granules.

Schomorl;s ferric ferricyanide stain reacted moderatly to the pigment granule of the mid gut in mature fishes. Pigmetns granules in different tissues of post mature fishes, however reacted stranaly to ferric ferricyandie stain.

Histochemical nature of lipofuscin pigments to carbol fuchsin was observed to some what different. Lipofuscin pigments in mid gut of mature fishes exhibited mid and noderate positivity to Long Zew - Neelsen;s carbol fuchsin method while in post mature fishes grnules were moderately recacted to carbol fuchsin in tissues.

The findings of the present study reveal significant realtionship of lipofuscin with the age of Channa Punctatus.

Lipofuscin pigment in supposed to be a parameter of age as age pigment of lipofuscin pigment progressively increased with age of the fishes.

In perematurе fishes cells were found with no pigment granules.

The first appearnce of lipofuscin pigments was observed at

the begining of mature age.

Pigment granules per cells and pigmented cells, increase with increasing of fish and vary at different age levles.

Homogeneous pigment granules were observed in mature age group.

Heterogeneous pigment grules were observed in post mature fishes. The maximum concentrationof pigment granules was observed in the gut cells of post mature fishes. There after mortility began in fishes.

It was observed that accumlulation of lipofuscin pigment became saturated in old age this stage supoosed to be the faltal age of fishes.

Differences between young and old pigment granules were clearly observed in mature and post mature fishes, and histochemical observations snowed that lipofuscin pigment granules had diffrent affinities with different stains at various age groups.

Table - 1

Summary of the Lipofuscin Pigmented Cell in Tissue with age.

Tissue	Age group	Pigmented cells/Volume Mean + S.E. %	Cells Pigmented
Midgut	Premature	-	-
	Mature		
	a	6 ± 0.18	30%
	b	8 ± 0.28	40%
	c	9 ± 0.86	45%
	d	10 ± 0.63	50%
	Post Mature		
	a	13 ± 0.41	65%
	b	14 ± 0.35	75%
	c	16 ± 0.52	80%
	d	17 ± 0.26	85%

Table - 2

Histochemical Nature of Lipofuscin Pigmented Cells in Tissue with age.

Age groups	Tissue	Stains		
		Nile blue A	Ferric Ferri cynide	Carbol fuchsin
1	Midgut	-	-	-
2		+++	++	++
3		+++	+++	++

+++ Strongly Positive
++ Moderately Positive
+ Mildly Positive
- Negative

CHAPTER - 6

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